NIH S10 Shared Instrumentation Grant

Resources for Submission

**For any questions on this document or on the S10 Shared Instrumentation process, please contact: April Weakley (aweakley@pennmedicine.upenn.edu) or Dr. Yale Cohen (ycohen@pennmedicine.upenn.edu)*

This document contains guidance for the NIH Shared Instrumentation Grant Programs. The document contains text from recent PSOM proposals that were funded through this mechanism, with PI approval. Figures have been removed to preserve readability and document length, and proposals may be viewed in full by requesting the document here. In addition to the references cited throughout this document, we also suggest the following resources which provide useful advice:

American Society for Cell Biology (ASCB):
How to Write a Shared Instrumentation Grant Application – and Get It Funded!

Agilent Technologies:
White Paper – Shared Instrumentation: How to Win the S10 Grant

In the case of any inconsistencies or discrepancies in any of these resources, please always refer to the NIH Shared Instrumentation Grant Program webpage, as is the official NIH source on these grant opportunities.

Section Headings Index

All page limitations described in the SF424 Application Guide and the Table of Page Limits must be followed, with the following exceptions or additional requirements:

For this FOA, there is no overall page limit for the entire Instrumentation Plan. However, there are specified page limits for each section of the Instrumentation Plan as described below. All tables, graphs, figures, diagrams, and charts must be included within the page limits for these sections. Applicants should make every effort to be succinct. It is expected that the length of the Plan's narrative will depend on the type of the requested instrument and the number of users. To be successful, an application does not have to reach the page limits described here. Note that you can click on the headings below to jump down to that section of this document.

- Introduction to Resubmission: 3 pages (if applicable) (Example: Goldman)
- Justification of Need: 9 pages in total (Examples: Goldman, Gupta, Litt, Sharp)
- Technical Expertise: 3 pages in total (Examples: Goldman, Gupta, Litt, Sharp)
- Research Projects: section must not exceed 30 pages in total. This section can be structured in subsections Research Projects of Major Users and Research Projects of Minor Users or subsections Specific Research Topics. The limit is 4 pages per each Major User's project; however, three or fewer pages are *strongly* recommended. The Research Projects of Minor Users subsection is limited to 4 pages. (Examples: Goldman, Gupta, Litt, Sharp)
- Summary Table(s): 6 pages in total (Examples: Goldman, Gupta, Litt, Sharp)
- Administration (Organizational / Management Plan): 6 pages in total (Examples: Goldman, Gupta Litt, Sharp)
- Institutional Commitment: 3 pages in total (Examples: Goldman, Gupta, Litt, Sharp)
- Overall Benefit: 3 pages in total (Examples: Goldman, Gupta, Litt, Sharp)
- Letters of Support and Other Attachments: Instrumentation Plan (in lieu of Research Plan section). The entire Instrumentation Plan (with the sections described below) must be saved as a single PDF file - named Instrumentation Plan - and attached via Other Attachments. Organize the Instrumentation Plan in the specified order (described below), starting each section with the appropriate section heading

(i.e., Justification of Need, Technical Expertise, Research Projects, etc.). Do not include links to websites for further information. Do not include animations/videos.

Introduction to Resubmission (3 pages)*:* Only in the case of a resubmission, include an Introduction describing the changes that have been made in response to comments in the previous review.

Goldman Example – Introduction to the SIG Proposal

This is a resubmission of an application submitted in May, 2015, for a commercial instrument, termed Multiple-parameter Fluorescence Detection Fluorescence Resonance Energy Transfer (MFD-FRET) that will acquire multiple signals simultaneously and enable detailed analysis of individual FRET events as sample molecules diffuse through a microscopic volume. The chief criticisms from the study section were threefold:

1. There was insufficient justification for this instrument. In this revised application
   a. We outline the unique strengths of the requested instrument and the limitations of current instrumentation available to our user group. Table A.1 in the proposal lists many of the attributes of the technology, MFD-FRET, above classical ensemble (bulk) FRET experiments.
   b. We provide a compelling rationale for the use of the proposed instrument by new users and outline over-sight and training for users.
2. The detection strategy of the instrument negates the advantages of single molecule detection. This is most certainly not true because various plots that combine the different signals, mentioned below and explained further in the proposal, enable homogeneous groups of molecules to be analyzed separately thereby resolving sources of inhomogeneity, such as incomplete labeling stoichiometry and exchange between structural states.
3. Another instrument, bearing similarities to the requested one, is already on the Penn campus.
   a. As correctly noted by a different referee, the instrument already at Penn would not fulfill the needs of our user group, but its presence is an advantage in regard to expertise and mutual sharing of materials and software.
   b. It differs in fundamental capabilities from the instrumentation being sought in this application.
   c. It has very limited availability outside the laboratory in which it is housed.

The following is a point-by-point response to the critical comments:

Justification of Need

a. **Detail about need for instrument features.** The basic confocal FRET instrument with pulsed interleaved excitation (PIE) is necessary to categorize and filter each FRET pulse according to whether it corresponds to a complex with one donor fluorophore and one acceptor. The main enhancements to this basic PIE configuration are the addition of two more detectors with polarizers to project the emission in each spectral channel onto two time correlated photon counters. This arrangement is necessary to remove the artifacts of dead-time and after-pulsing in the detectors by correlating pulses from the two detectors per channel (Windegren et al., J. Phys Chem. 99:13367, 1995). By separating the emission using polarized beam splitters, rather than 50:50 prisms, fluorescence anisotropy is also obtained “for free”, enabling the orientation factor, $\kappa 2$, to be estimated directly. These points and the necessary features of the equipment are more carefully explained in the application and their requirement in the user projects.

b. **Use by projects of the special features, such as rotational anisotropy.** All of the projects that attempt to obtain quantitative distance estimates by FRET efficiency need PIE, donor nanosecond fluorescence lifetime, and polarized detection to separate (filter in) those molecules properly labeled with single donor and single acceptor molecules and to estimate κ2. This point is explained further in the application and in the user project descriptions.

c. **The method isn't truly single-molecule, since many FRET pulses need to be aggregated in order to interpret the data, e.g. it's really an ensemble technique.** Plotting FRET efficiency (EFRET) against stoichiometry (Fig. 1A of the application), EFRET against donor lifetime (Fig. 3A and 4), and EFRET against rotational anisotropy (Fig. 3A) very effectively separates out different species in the sample, for instance different distances or different probe stoichiometries. We call the separate peaks sub-ensembles which are analyzed on their own. Thus the method achieves removal of inhomogeneities among individual particles as in a single molecule experiment. This separation of species is achieved with the advantage that no immobilization to a microscope slide surface is required. For certain time domains, individual reaction trajectories can be monitored, as in true single-molecule experiments, although diffusion must be slowed in this case by attaching the sample to a vesicle or bead (Fig. 3). These points and the various regimes of dynamics are described in more detail in the revised application.

d. **Other instruments on campus could be used in collaborations**. The other FRET instruments are much less capable than the one requested. Moreover, they are heavily used by those laboratories and configured for special purposes. The enhancements requested here are beyond just additional wavelengths; they include markedly enhanced capabilities and features that enable access to more far more users – both be-cause of its capabilities and the staff dedicated to assist new and experienced users.

e. **The instrument is an enhancement in technical capability, but the limitations in present capabilities and why this level of instrument is required were not described.** In this revised application, we emphasize that the instrumentation being sought is not an enhancement of current FRET capabilities in the user labs, but rather an entirely different research approach. Existing FRET instruments are described in the revised application. Other techniques either 1) measure average FRET in an ensemble of millions of molecules in a cuvette, and are therefore susceptible to inhomogeneities in the sample, or 2) they require immobilization of the sample and a major investment in experimental time to make enough measurements for statistically valid conclusions. MFD-FRET is much easier than single molecule FRET microscopy, it yields good statistics in short order, and obviates immobilization. Thus it is accessible to a much wider community of investigators, while requiring much less experience and effort. Experiments with high reliability and success rate may be completed in a single day.

f. **Some users are not currently undertaking FRET experiments**. Some of our users are experienced in macro-FRET and single molecule FRET. But having this instrument readily accessible will transform their research by making this very straightforward method available. Others who have been reluctant to use FRET because they lack experience in optical methods or microscopy, or because they fear the checkered reputation of FRET and

all its uncertainties, will be pleasantly surprised by the ease of use and the clear results. Thus, an instrument configured as requested and as a facility with expert staff, will benefit the work of many investigators.

g. **Extensive molecular dynamics experience is needed**. As explained in the proposal, the software sup-plied with the instrument and in the public domain is user friendly and does not require high skill or expertise. For the most rigorous measurements where small errors in distance between the labeling sites could affect the conclusions, more detailed molecular dynamics calculations will be advisable. We have many experts and groups skilled in this type of simulation on the Penn campus; they struggle for lack of data from the type of experimental instrumentation we seek, not for lack of simulation experience or computational resources!

2. and 4. Administration and Technical Expertise

a. **The previously identified technical director has more EM and biochemistry experience and not single molecule**. The facility manager identified in this revised application has extensive, published experience in single molecule technology and in managing and maintaining multi-user instrumentation. He has experience in burst-mode FRET experiments and will receive additional training from Dr. Claus Seidel in Düsseldorf, Germany. Please see Dr. Seidel's letter of support included with this application.

b. **Staff members do not have experience in managing a multi-user facility.** The facility manager identified in this revised proposal is already managing shared instruments in the Pennsylvania Muscle Institute very effectively.

c. **Complex experiments many not be amenable to the wider group.** Compared to other instrumentation, and other ways of obtaining this kind of information, MFD-FRET experiments are very simple to perform. Design and interpretation does require considerable expertise, but that expertise is readily available.

d. **Estimated usage too high.** We have reduced the estimates to provide time for development, maintenance and new users.

e. **No description of AUT or how it was calculated.** The estimated user times are based on the expected complexity of each project's experiment and how extensively MFD-FRET experiments and how many variations would be necessary to reach conclusions.

f. **Training plans not well defined**. The facility manager will determine whether users are qualified to operate the instrument, or require supervision (e.g. students). There is little that an inexperienced user can actually damage, but there are safety and interpretation issues that need explicit training.

3. Research Projects

a. **Project descriptions are short and lack strong rationale for FRET experiments.** We have expanded the project descriptions and their objectives with a focus on features and advantages of the requested instrumentation for each project.

b. **Some projects did not seem feasible.** The range and regime of each project for diffusion-based pulse-mode FRET measurements have now been explained.

c. **User group may be narrow due to complexity of the experiments and interpretation**. We have an aggressive, well funded user group that is well aware of the limitations of existing instrumentation, and that is eager for access to the capabilities of the proposed instrumentation.

d. **Insufficient explanation why current instrumentation is not sufficient.** See response under Justification of Need above.

e. **Details of the experiments are not described.** The projects are highly feasible, but at this time most of them are conceptual in nature because they simply cannot be done with existing instruments. Indeed, there are insufficient instrumental resources at present to collect preliminary data and outline specific conditions for the proposed experiments. Until an instrument becomes available, prospective users cannot be expected to budget effort for labeling samples or for preliminary experiments for instrumentation that does not presently exist.

f. **Some users have not demonstrated fluorescence or FRET experience.** See response under Justification of Need above.

5. Institutional Commitment

The School of Medicine committed support for the facility manager and service contracts for the useful life of the instrument, but the amount of money needed was not stated. The letter of support and commitment from the Associate Dean and Chief Scientific Officer of the School of Medicine, University of Pennsylvania, outlines entirely reasonable levels of support for the facility manager, service contracts, and budgetary shortfall.

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Continue on to Justification of Need

***Justification of Need (9 pages) Instructions*:** Name the requested instrument. Compare performance of the requested model with other similar instruments available on the market. Justify the need for specific features and special accessories of the requested instrument. Each such accessory must be utilized by at least three Major Users. Explain why the chosen model and its manufacturer are the most suitable for your user group. Preliminary data are not required, though if feasible, you may include preliminary data to justify your choice. Provide an inventory of similar instruments existing at your institution, neighboring research institutions, or otherwise accessible; describe why each similar instrument is unavailable or inappropriate for the proposed research. If similar instruments are listed as "unavailable," add a letter to the Letters of Support section from the instrument manager explaining why the instrument is not available to your user group.

- Include specific documentation on the current usage and downtime of each of these existing instruments in annual hours and a realistic estimate of the projected usage for the requested instrument. You may use tables to clarify the presentation. Tables included within this section will count towards the specified page limit.
- For the requested instrument, define and justify the Accessible User Time (AUT) which is the number of annual hours the instrument can be used for any research purpose. AUT hours may be limited by the times an instrument operator is available (if an operator is required), site or building access schedules, estimated or scheduled maintenance, start-up and standardization, and any other factors that take time away from the use of the instrument. Justify the AUT for the proposed instrument based on the individual situation at the applicant institution. AUT for the same instrument may differ among different institutions.
- **Guidelines:** As adapted from the ACSB, the Justification of Need section, more than any other, allows you to be creative in selling and communicating the need for the requested instrument. It includes:

1) This section should start with a single brief paragraph summarizing the scope of the proposal in terms of the user group, instrument, cost, and instrument capabilities. It is essentially a brief synopsis of the Foreword/Summary for reviewers.
2) A brief history of the core facility/entity in which the instrument will be housed
3) One paragraph should describe the requested instrument and a brief historical perspective about the development and evolution of the instrument and/or technology. This is where you will convince the review panel that you understand the technology and appreciate the pros and cons of the proper use/application of the instrument – show's you have the perspective and are a knowledgeable expert.
4) A statement about the rationale for selecting the instrument and its importance to the research
5) A comparison of the requested instrument with other similar, commercially available systems
6) A discussion of access to existing equipment and why that equipment is either unavailable or not suitable for the needs of the users/potential users
7) Last, a summary of the proximal inventory of similar systems that might have been used but cannot be for one reason or another. Either provide letters from the owners or core directors of the other instruments that attest to the reason these instruments are unavailable to support this.

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Goldman Justification of Need

Goldman – Justification of Need

A.1. Diffusion-based Single Molecule Multi-Parameter Fluorescence Energy Transfer

A broadly applicable technology for obtaining reliable and accurate distance, occupancy and dynamics measurements over a wide range of timescales in macromolecules by fluorescence resonance energy transfer (FRET) has become commercially available. This technology represents a major research opportunity for a large number of investigators at the University of Pennsylvania, many of whom are actively working with advanced optical microscopy and computational structural dynamics. The classic FRET experiment involves two fluorescent probes, one with an emission spectrum (termed the donor) that overlaps the excitation spectrum of the other (the acceptor). When the two probes are within ~2 - 8 nm of each other, photoexcitation of the donor results in the non-radiative transfer of energy to the acceptor. The efficiency of transfer is related to the relative spatial orientation of the donor and acceptor, and the 6th power of the distance (*r*6) between them. Thus, measurements of FRET efficiency yield information about distance in a range that is relevant to the function of many proteins, nucleic acids and macromolecular assemblies.

Theodor Förster extensively characterized this phenomenon in the 1940's1 and it has been used in hundreds of biophysical and biomedical studies in the interim2,3. Despite its popularity, FRET measurements are subject to many artifacts and uncertainties when performed in classical ensemble (cuvette) experiments3,4 due to non-stoichiometric labeling of the sample components, contaminants, anomalous photo-physical behavior of the probes, unknown rotational mobilities, and averaging over static and dynamic inhomogeneities in the samples. Because macromolecules may adopt many conformations as they perform their function, inhomogeneities may represent normal functional variations or sample degradation. These problems reduce the quantitative reliability of FRET signals, but are largely eliminated with a new approach termed diffusion-based single molecule Multi-parameter fluorescence detection FRET (MFD-FRET).

In addition to distance and occupancy signals (association of binding partners), MFD-FRET technology has recently evolved into a very facile and experimentally straightforward method for measuring the kinetic parameters when the experimental sample contains more than one structural species. This situation is very common among active macromolecules undergoing enzymatic or association/dissociation reactions. Exchange on the nanosecond to millisecond time scales are quantifiable as will be explained below (Fig. 4).(Figure 1)

This proposal requests funds for a commercial instrument that will acquire multiple fluorescence parameters simultaneously and enable detailed analysis of individual FRET events as sample molecules diffuse through a microscopic volume in which they are excited and detected. Fig. 1 shows a schematic of a MFDFRET instrument. Very low sample concentrations (10 – 50 pM) lead to molecules diffusing through the diffraction-limited detection volume one at a time. Pulsed Interleaved Excitation (PIE, also termed Alternating Laser Excitation, ALEX) alternatingly excites the donor and acceptor fluorophores. Three sets of photon counts become available by time correlated single photon counting (TCSPC): *I*dd, *I*ad, donor and acceptor emission under donor excitation and *I*aa, acceptor emission while directly excited. FRET efficiency calculated from the emission intensities and decay rates, while artifacts due to contaminants, scattering, photo-bleaching or absence of the acceptor are distinguished according to the fluorescence emission when the sample is excited by the two pulsed laser wavelengths (panel C). Panel D shows a map of stoichiometry (S) *vs*. FRET efficiency (EFRET) in which three species are clearly distinguished: (1) donor only or contaminant particles that can be ignored (S~1), (2) properly labeled (1 donor and 1 acceptor) molecules with S = ~0.5 and relatively low FRET, and (3) properly labeled molecules with relatively high FRET. This experiment illustrates a major advantage of PIE excitation and single photon counting: the peaks in the 2D plots (Figs. 1, 3, 4) are analyzed separately, thereby resolving various species or conformations in the sample. The different groups of molecules are termed sub-ensembles and represent relatively homogeneous species or structures that would be artifactually grouped in a classical (cuvette) FRET experiment.

The instrument we are seeking is illustrated in Fig. 2. It is equipped with two additional detectors that are polarized so that kinetic rates and fluorescence anisotropy decay (rotational motion) may be measured as well.

As a result of coordinated analysis and correlations between the channels, each FRET event is characterized in an 8-fold parameter space (Fig. 2): fluorescence anisotropy (rotational diffusion), fluorescence lifetime (probe environment or energy donation), intensity (stoichiometry), detection time (related to diffusion coefficient), excitation spectrum, emission spectrum, fluorescence quantum yield (environment), and distance between the two fluorophores6. Two correlated detector channels are required to eliminate effects of detector dead-time (minimum interval between registered photons) and artifactual after-pulsing9,10 and when each spectral channel's emission is split by polarizers, rotational motion (anisotropy decay) is obtained in the same group of signals. This approach is known as Multi-Parameter Fluorescence Detection (MFD). Analysis of these signals enables identification and characterization of individual species present in the population of molecules, and powerful insight into the interconversion rates between these species. The single-molecule approach permits correction for labeling stoichiometry, discrimination among heterogeneous species, and quantifying internal structural dynamics of the macromolecule. Rotational and lateral diffusion rates indicate complex formation between ligands. Ultimately, FRET measurements are interpreted as the distance between donor and acceptor, exchange rates among several conformations. The distances between points where the donor and acceptor are attached to the macromolecule (rather than inter-fluorophore distances), are given either by taking account of the range of fluorophore accessible positions7 or in a model generated by molecular dynamics simulation. Please see Sect. B.5.c regarding post-processing analysis. (Figure 2)

Two additional powerful capabilities of MFD-FRETare illustrated in Fig. 3. Although the S vs. EFRET plot in Fig. 1 (and in Sections C.1.a and C.1.c) is the basic display for PIE MFD-FRET, several other displays emphasize other aspects. Distinct molecular species, such as different conformations or bound and unbound ligands are easily and more sensitively distinguished (e.g. Fig. 1D) in plots of Donor (*F*D) / Acceptor (*F*A) fluorescence and donor fluorescence anisotropy (*r*D) vs. donor lifetime (t). In Fig. 3A, the investigators7 purposefully mixed DNA duplexes of length 5, 11, and 19 base pairs labeled at their two ends and DNA duplexes with the donor fluorophore only. The four species present simultaneously in the sample are easily distinguished and quantified. The red curves show expected relationships (Perrin equation).

Although the individual FRET events detected as an individual molecule give rise to the 8 measurement parameters, the peaks in MFD-FRET plots come from several thousand individual events. These statistically valid groups are thus, in themselves, not single molecule data. They discriminate among structural species (and other inhomogeneities) in the sample and each peak can be analyzed on its own by selection along the S or EFRET axes. Such groupings are termed sub-ensembles and are greatly preferred for interpreting the experimental results than the classical average FRET efficiency, measured in a cuvette, or fluorescence correlation spectroscopy, measured at higher than single-event concentrations, when several molecules are in the detection beam simultaneously8.

The rates of exchange between species may be obtained by a temporal analysis of the 4 intensity signals during occupancy in the detection beam. A wide range of kinetic constants are made available (100 s-1 – 1,500 s-1, ref 5) by tailoring the diffusion time or detection volume. For instance, diffusion was slowed in the experiment of Fig. 3B by tethering the sample to a lipid vesicle. Other routes to adjusting diffusion rate and consequent occupancy in the detection beam are encapsulation in lipid vesicles or reverse micelles (See project C.2.g (Wand)), or tethering to polymer beads. Overall, a great number of useful and convenient signals may be achieved using the MFDFRET instrument we are requesting.

Another cutting-edge experimental outcome is evidenced when the sub-ensemble peaks in the 2D MFD-FRET plots fall away from the curves based on static lifetime and efficiency expectations (the red curves in Fig. 3). Fig 4 shows an example of this situation in which the sample (fluorescent labeled syntaxin 1) contained two rapidly exchanging, “open” and “closed”, conformations. The FRET peak in the 2D histogram in Fig. 4A tracks along a theoretical (green) line giving the expected FRET and donor lifetime for two rapidly exchanging species. When the dynamics for exchange are faster than the ms occupancy in the detection beam, then fluorescence correlation spectroscopy at higher concentrations (nM) can be measured. In this case more than one fluorophore is located the detection volume. Auto-correlation and cross-correlation of fluctuations of the acceptor and donor intensities as the two lasers are alternated provides data for the exchange rates (Fig. 4B). Although this type of experiment is not the main thrust of the experiments planned on the requested instrument, the FCS-FRET technique is performed on the same instrument and will be available to our users. It shows the power of the general method for analysis of macromolecular structural dynamics we will be able to supply.

The requested instrument will provide a major new source of experimental capability to the UPenn research community that is not available otherwise. As delineated in the example projects below, novel data, not otherwise accessible, will be obtained in a quite quickly and straightforwardly manner. The ability of this technology to remove most uncertainties from FRET distance measurements, simultaneously detect the numbers of bound species, and the kinetics of exchange between intra-molecular FRET structural states and inter-molecular changes due to ligand binding and exchange are not accessible by other means. Very few groups around the world have the advanced capabilities provided by this tool and its associated analytical

methods. But for the experimental user, they are very small experimental increments enabling major structural dynamics advances. Supplying this capabil-ity to the UPenn community will accelerate and enhance many ongoing research projects as explained in the example given below. Thus, the justification for the request is to enable improvement and acceleration of our funded projects and to enable new lines of investigation, not previously deemed to be feasible, but will be quite straightforward within this facility.

Table A.1 lists some of the problems commonly associated with FRET experiments and the routes to their elimination or correction using MFD-FRET.

Common Problems in FRET Experiments	Solutions Obtained by MFD-FRET
1. Incomplete or mixed labeling stoichiometry	PIE excitation separately quantifies relative number of each fluorophore in each FRET pulse (Fig. 1).
2. Local quenching of donor or acceptor	Expected position on 2D plot of FRET vs. donor lifetime $\tau_{D(A)}$ (Fig. 3A, upper 2D plot) excludes these effects.
3. Differences in rotational mobilities or κ^2 changes	Expected position on 2D plot of $\tau_{D(A)}$ vs. rotational anisotropy (*r*) (Fig. 3A, lower 2D plot) excludes these.
4. Flexibility of linked fluorophores	Proper weighted averaging of FRET signals $E(\langle R_{DA} \rangle)$, which is not the same as $\langle E(R_{DA}) \rangle$.
5. Component FRET distributions broadened by shot noise	Deconvolute peak width according to photon counts.
6. Rapid exchange between conformations during transit in detection beam	FRET vs. $\tau_{D(A)}$ plot shows deviation. Kinetics accessible by FRET-FCS experiments on the same instrument.

References

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12. Suren Felekyan, Hugo Sanabria, Stanislav Kalinin, Ralf Kühnemuth, Claus A.M. Seidel. (2013) Analyzing Förster Resonance Energy Transfer with Fluctuation Algorithms. Methods in Enzymology 519: 39-85.

A.2. Personnel of the MFD-FRET Facility.

Yale E. Goldman, MD, PhD, PI and Scientific Director
Dr. Paul H. Axelsen, MD, Co-PI and Data post-processing Director
Dr. Serapion (Ionas) Pyrpassopoulos, PhD, Technical Facility Manager

Oversight: An Internal Advisory Committee (IAC) has been assembled to develop strategic plans, evaluate service and progress and report to the Departmental Chairs of Physiology and Biochemistry and Biophysics supporting the facility. Dr. Kevin Foskett, Chairman of Physiology, School of Medicine, will serve on and Chair the IAC. The other members are Drs. Elizabeth Rhoades, Dept. of Chemistry, Dr. Louis Soslowsky, Associate Dean for Core Facilities, Dr. Sergei Vinogradov, a probe and optics expert in the Dept. of Biochemistry and Molecular Biophysics, and Drs. Axelsen and Goldman, ex-officio.

A.3. Equipment in the Facility

We request funds to purchase a PicoQuant MicroTime 200 Fluorescence Correlation Spectrometer/Microscope fitted out for diffusion-based single molecule MFD-FRET. This is a highly modified Olympus IX73 inverted microscope on a stable base and a Main Optics Unit that sup-plies special purpose laser fluorescence excitation and detection of individual molecules diffusing through the confocal excitation/detection point spread region. A partly cut-away image of the instrument is shown in Fig. 5. We have chosen this instrument due to its proven reliability, the available options to enhance it by adding the two extra detector channels, as explained else-where, and the software available from our expert advisor and developer of much of the technology, Dr Claus Seidel, Düsseldorf Germany. Please see Dr. Seidel's letter of support and offer to help train our personnel.

The main subsystems required are 1. Excitation system, 2. Microscope system, 3. Main Optical Unit (MOU) (incl. confocal unit and two detectors), 4. Electronics and system software, 5. Detector Extension Unit (DEU) (for two additional detectors), 6. Additional components, including optical filters, mechanics and controls, 7. Analysis software, 8. Analysis Computers. All of these items are essential for configuring the instrument to pro-duce quantitative and reliable distance and dynamics data.

1. Excitation System PDL 828-L "SEPIA II" Mainframe for PC controlled oscillator / burst generator / sequencer, picoseconds diode, 8-channel laser driver. Includes laser optical heads and requisite mounting hardware, optics and filters for 440 nm, 485 nm, 532 nm, 560 nm and 640 nm excitation lasers. The excitation system is based on picosecond pulsed diode lasers and consists of individual laser heads, sets of suited optical filters, the specialized short-pulse laser driver that controls the laser repetition rate and output power each laser, timing of the interleaving wavelengths and coupling into a polarization maintaining optical fiber through a Laser Combining Unit (LCU). The laser drivers generate a standard and uniform driving signal and feature easy-to-use controls for pulse width, repetition frequency, and laser power level. The wavelengths were chosen form the most common FRET probe pairs, Alexa 488/Alexa594, Rhodamine110/Cy3, Cy3/Cy5 and GFP variants CFP/YFP and venus/Cerulean.
2. Microscope Research grade inverted Olympus IX 73 microscope with manual condenser unit, manual fluo-rescence filter cube revolver, binocular eyepieces, special right side port for confocal optics and hardware

up-grade for 3D piezo imaging synchronized with software. The microscope is configured as inexpensively as possible with many manual controls where software drivers are not necessary. The piezo scanning unit is required for avoiding focus drift and for beam diagnostics.

3. Main Optical Unit (MOU) containing MOU excitation unit, apochromatic corrected collimation (4 x 0.16) for fiber output, MOU basic confocal unit with dichroics and filter wheel, MOU beam diagnostics, MOU bypass op-tics, MOU / DEU Opto-Mechanics for addition of detectors #3 and #4, and 2 Single Photon Counting Modules (SPADs). This is the enclosed box to the right of the microscope in Fig. 5, containing the lasers and detectors. It is the main, integrated optical component that enables diffraction limited, pulsed interleaved excitation (PIE) of pairs of fluorophores for MFD-FRET with fully corrected distance and dynamics measurements, either at single molecule, pulsed concentrations (pM) or higher (nM, FCS) fluctuation analysis.

4. Electronics and System Software consisting of HydraHarp 400M picosecond event timer and TCSPC module, four channel TDC modules for HydraHarp 400, Host PC system, and SPT64-1+2 "SymPhoTime 64" analysis software. Fig. 6C shows the user inter-face for the HydraHarp driver/ SymphoTime analysis software, which is convenient and user-friendly.

5. Detector Extension Unit (DEU) consisting of DEU frame, MOU /DEU Opto-Mechanics for 2 additional detectors, Single Photon Counting Modules (SPADs) #3 and #4. Four detectors are required for correlation to remove dead-time and after pulsing artifacts (refs. 9 and 10 above). As mentioned, separating the emission by polar-ized beam splitters makes anisotropy and ns anisotropy decay ac-cessible at the same time as removing these detector artifacts. Thus we will have firm estimates of κ2, normally a major unfounded assumption in classic FRET measurements.

6. Mechanical and Optical Components. Many factory-assembled opto-mechanical items and filters are required for system integration.

7. Two additional licenses for SPT64 SymPhoTime 64 analysis software are necessary for users to analyze their results while oth-ers are conducting experiments.

8. Two computing intensive PC workstations for off-line anaylsis will smooth the data work-flow and enable users to obtain results very quickly to interpret them and plan further experiments.

A.4. Usage of the Facility

We are developing this facility for all Penn Faculty and labs. This type of equipment is new and is not currently available at Penn. As noted above, 12 investigators have immediate need for high quality FRET distance, stoichiometry and dynamics measurements. The current major users come from the departments of Physiology, Pharmacology and Biochemistry and Biophysics. We anticipate that the users will fall into two categories: (1) Those who will go through training on the machine and then continue to use it independently and (2) those who have trial experiments that are performed by facility staff.

A.5. Other FRET Instruments on Campus and their Limitations

There is no comparable multi-parameter diffusion-based FRET instrument currently on the Penn campus. Dr. Feng Gai, in Chemistry, has a much simpler confocal fluorescence correlator on which we obtained the FRET recordings shown later in Fig. 8B-E. There is one 514 nm continuous laser, so that instrument is not able to perform pulsed interleaved excitation, measure lifetimes or anisotropy decay. Dr. Elizabeth Rhoades in Chemistry has a PicoQuant MicroTime 200 instrument with some capabilities that overlap with the present request. Please see her letter explaining why we cannot make use of that equipment for the current purposes. The main reasons are that it is configured specially for her own studies, it does not have the 4 detector channels required for fully quantitative FRET distributions, and polarized anisotropy detection to estimate κ2, and it does not have the laser wavelengths we need. For the highest quality FRET recordings that can be converted quantitatively into distance estimates, the anisotropy signal is crucial to document mobility of the

fluorescent probes and rotational mobility, thereby providing an experimental κ2 value. For detection of dynamics of ex-change between fluctuating species in the sample, the four detectors are also necessary to eliminate artifacts due to detector dead-time and after-pulsing. Dr. Rhoades' instrument is also under heavy use by her group.

Many classical spectrofluorometers are available in labs of the present user group and in other locations. FRET may be measured in these fluorometers, but classical ensemble FRET averages over the range of molecular species and conformations and thus cannot distinguish among these different populations in the sample. In most of the projects proposing to use the requested instrument, some amount or major amounts of natural and artifactual inhomogeneities are expected, for instance from fluctuations between conformations. The technique of MFD-FRET distinguishes among these populations by various 2D plots, shown in Figs. 1-4: EFRET against probe stoichiometry, against donor excited state lifetime, and against rotational anisotropy. The peaks in the contour plots generated from these plots are termed sub-ensembles and contain largely homogeneous groups of molecules that can be analyzed together. This powerful capability is not available in standard laboratory fluorometers.

The PI, Dr. Goldman, has 4 very specialized single molecule microscopes in use for tracking position of molecular motors, measuring rotational motions in motors and protein synthesis elongation factors (EFs), and optical trapping of cargos in cells and in vitro samples. One of these microscope is largely used for FRET measurements on the EFs and fluorescent tRNAs. Please see references in the biosketch for a sampling of these studies. These microscopes are not suitable for the presently proposed projects for a several reasons: 1) the samples must be immobilized on the surface, necessitating considerable effort to maintain and check functionality, 2) dynamics are generally limited to camera frame rates, ~30 s-1, unless smaller areas of the sample are viewed, which limits data collection, 3) although preliminary experiments and trials by colleagues are al-ways welcome, the single molecule microscopes are in heavy use for the PI's own NIH funded research and thus are not readily available for large numbers of substantial collaborations in unrelated scientific areas. The MFD-FRET technique with labeled diffusing molecules is a much easier experiment to perform and yields many of the advantages of single molecule experiments, such as resolving groups of conformations or species in the sample and quantifying dynamics of exchange between structural states over a faster time scale.

Thus an independent facility, with the features requested here, and located in the School of Medicine is the only way to fulfill the needs of a rather large immediate user group and others with as yet unanticipated needs.

A.6. Our reasons for choosing the PicoQuant MicroTime 200 Fluorescence Correlation Microscope

As listed in the biosketch, our lab and research group has had extensive, successful experience building high-end state-of-the-art microscopy and biophysical equipment, for instance for laser photolysis of caged ATP, nanometer tracking of individual fluorophores and single molecule polarized TIRF (polTIRF) microscopy for high-speed rotational motions (please see biosketch). This is the only way to proceed if a method is utterly new and not available from commercial sources. But the first iterations of these instruments and their operation and analytical routines are not made to achieve ease of use. Here we are planning to open MFD-FRET to a community of effective biophysical workers, many of whom have not worked in the single molecule regime or with high end, home-brew equipment. The physical instrumentation and especially the software in a commercial instrument, once a technique is established, are much more stable, user friendly, and amenable to concise and complete training. Thus for the present goal of distributing these powerful methods to a broader user group, the extra expense of a commercial instrument is well worthwhile.

There are two main competing companies in this area of fast-pulse time-correlated single photon counting (TCSPC) hardware, Becker and Hickl and PicoQuant, both headquartered in Germany. We have used OEM equipment from Becker and Hickel and one of their Principles, Dr. Wolfgang Becker visited our lab and revealed a special trick to "fool" their hardware into cataloging the polarization state of photons for our high-speed polTIRF instrument. Becker and Hickl have focused on upgrades to commercial confocal instruments for fluorescence lifetime imaging (FLIM), not single molecule MFD-FRET. It would be possible to assemble a MFD-FRET system with their TCSPC modules, but the software would not be nearly as convenient, the optics special order, and overall operation and maintenance would be similar to one of our lab-made instruments with all the inconveniences noted. Thus the PicoQuant system, purposefully designed for the experiments we are targeting, with convenient intuitive software and many other groups around the world satisfied with its perfor-mance, is the clear choice. We have configured the instrument with very high performance and with most of the available options that accompany fully quantitative MFD-FRET experiments because our goal is to obtain the most tightly quantified FRET efficiency values, leading to dynamics and distances that are the most reliable state of the art data available and still maintain ease of use by the local user community.

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Gupta – 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 (°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°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.

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Litt – Justification of Need

The requested instrument is a BlackRock Microsystems 512-channel NeuroPort recording system with integrated 96-channel CereStim stimulation system. The instrument will be used to record and stimulate electrodes implanted in human patients with epilepsy. There is a growing number of research studies investigating neuroscience questions using epilepsy patients implanted with grids of electrodes that sit on the cortical surface (electrocorticography, ECoG) and depth electrodes that penetrate into the brain with recording contacts spaced at regular intervals along the shaft (sterotactic EEG, sEEG). Taken together, both techniques are referred to as intracranial EEG (iEEG).

The BlackRock system will be located within a 12-bed Epilepsy Monitoring Unit (EMU) locatedin a brand-new hospital, known as The Pavilion at the Hospital of the University of Pennsylvania (opening 2021). In our EMU, we will use parallel systems for clinical monitoring and research studies.For clinical monitoring, a Natus system (purchased by the hospital) will continuously route iEEG datato a clinical reading room, where epileptologists and technicians review the iEEG data, collected andstored at low sampling rates, for pathological activity. For research purposes, the BlackRock systemwill record from the same electrodes at much higher sampling rates with greater flexibility. This parallel setup ensures that clinical monitoring continues uninterrupted independent of research activity. Three capabilities of the BlackRock are particularly critical for research. First, the ability to record from 512 electrodes simultaneously. This allows studies in which the patient is implanted with100 to 200 clinical electrodes and an additional complement of research electrodes, often smaller and located between the clinical electrodes. Second, high sampling rates are necessary for researchexperiments in which single neuron activity is recorded, such as with Behnke-Fried electrodes that contain microwires at the terminus of the sEEG electrode. Third, stimulating iEEG electrodes allows causal brain-behavior relationships between activity in small populations of neurons to be established, and closed-loop stimulation can be used to control or normalize brain function. There are only a handful of vendors producing equipment with these capabilities. Blackrock is the most widely used of these systems and has been purchased for NIH-funded human studies at 35 differentacademic centers.

The University of Pennsylvania has long been on the cutting edge of iEEG research. in 2011, PI Litt received an S10 for the purchase a Neuralynx system for our EMU. As a result of the 2011 S10, our iEEG research program grew dramatically, allowing us to publish ~100 peer-reviewed journal articles. The Neuralynx is now outdated and has been retired from the EMU, but is still usedfor animal recording studies. From 2014 to 2018, Penn participated in the Defense Advanced Research Projects

Agency "Restoring Active Memory" (DARPA RAM) program (S10 major user Kahana was the Project PI and S10 major user Davis was the site PI). The DARPA RAM apparatusincluded BlackRock equipment. While some of this equipment is still operational despite its advancedage, it suffers from a number of limitations that make it unsuitable for installation in our new EMU. First, it has only a quarter of the recording channels of the requested equipment (128 *vs.* 512) meaning that there is not sufficient recording capacity to record the number of electrodes that we now typically implant. Second, the stimulation hardware was custom-built with Medtronic specificallyfor the DARPA RAM project and is not being supported by either Medtronic or BlackRock. This makes it unsuitable for our ongoing research projects that require the state-of-the-art CereStim hardware. However, our experience with NeuraLynx and Blackrock demonstrate that there is a verystrong need for the requested equipment, and a community of proficient, well-funded investigators who will utilize it to its maximum potential.

To jumpstart our iEEG research program in the new hospital, we have devoted considerable effort to the design of our new EMU. In most facilities, recording equipment is located on mobile carts that must be wheeled into the patient's room before research can commence, and then removed again at the termination of each experiment to allow for data download and analysis. This is inconvenient for both patients and researchers, and frequent connection and disconnection of the electrode connections can degrade data quality. Instead, our new EMU has been constructed with a dedicated research room to allow experimenters to analyze data and prepare for experiments without interfering with patient care. The dedicated research room is 400 square feet, with workspace for researchers from different groups and a computer cluster for real-time data analysis. The room contains cable conduits connecting the research room to each of the 12 patient rooms to allow a fiber optic connection between the BlackRock headstage and the NeuroPort. Each patient room is also equipped with blackout curtains for better control of patients' visual environment (important for studies of memory and perception).

Epilepsy patients undergoing intracranial EEG (IEEG) for presurgical evaluation are typically hospitalized for up to several weeks, while seizures are recorded, localized, and epileptic networks are interrogated with electrical stimulation during surgical planning. This provides an unprecedented opportunity for neuroscientists to explore functional networks in brain in health and disease. Traditional electrode systems used in standard clinical practice, are largely unchanged in their basic design since the 1940s. These consist of 0.5-1 cm diameter metal discs that sit on the surface of the brain, or penetrating depth electrodes containing 6-12 individual cylindrical metal contacts, ~1mm in diameter and 0.5 cm long. Advances in electrode design have allowed for the augmentation of these standard arrays and the development of custom arrays for measuring neural activity in the human brain with unprecedented precision, including single and population activity. For example, recent research by our group and others demonstrates that higher frequency activity (>70 Hz), particularly that recorded by microelectrodes on the scale of 40 microns, access a rich spectrum of physiology not appreciated in human recordings prior. Fast computers capable of sampling, storing and processing data streams from these electrode arrays which can reach Terabytes in length, give us the ability to sample and begin to understand multi-scale electrophysiology in humans. They providea gateway into functional network physiology in health and disease in humans that is otherwise not accessible to researchers and clinicians. In recent years this work has been augmented by high resolution and responsive electrical stimulation, to both map networks causing seizures, but also to explore somatosensation, audition, memory, mechanisms motivating behavior, motor control, smell and a variety of other areas, by investigators at the University of Pennsylvania and elsewhere. Penn’s large group of investigators in systems neuroscience, situated in close proximity to one of the country’s

finest and busiest academic epilepsy centers, provides a unique opportunity for collaborative research on human electrophysiology, that has grown explosively over the past 10 years.

The University of Pennsylvania provides a unique research environment, particularly for clinical translation in the neurosciences. With a compact campus housing over 160 faculty doing research in the neurosciences, at the center of which is one of the country's most prominent and busy academic medical centers, the opportunity to translate basic science in human devices and therapies is extremely rich. At the core of this proposal for the requested equipment, is a group of researchers all of whom do research on conditions extremely relevant to human health: epilepsy, cognition, vision, olfaction, consciousness, and hearing. Our research spans multiple basic science laboratories as well as clinical centers at Penn including Neurology, Neurosurgery, Otolarygology, Neuroscience, Psychology, Psychiatry and Engineering. Our group is at the center of national and international collaborations in which we collect high resolution data from animals, as well as from humans, sharing our data worldwide through online platforms developed by our team (www.ieeg.org).

The Penn Epilepsy Center is a very active group consisting of 10 full-time epileptologists at the Hospital of the University of Pennsylvania and 3 epilepsy neurosurgeons. Together our groups perform approximately 35-40 epilepsy implants and over 100 epilepsy surgeries per year, including resections, laser ablations stereo and grid/strip intracranial electrode implants, and implantations of open loop (thalamic stimulation) and closed loop (NeuroPace Responsive Neurostimulation System) surgeries. These numbers are growing steadily and will increase markedly when our new 12 bed unit opens in the Pavilion. Our translational research group continues to grow our expertise and research mapping epileptic networks, particularly using high frequency oscillations, multi-unit activity, and high-bandwidth electrophysiology. In addition, our group is extremely active in developing new high-resolution implants for neural recording, modulation, control, and neuroprosthetics for hearing, vision, sensation, movement and memory (more details in research projects below). This work includes active collaborations with John Rogers at Northwestern University, Flavia Vitale at Penn and Jonathan Viventi, at Duke University. An important new development is the addition of Dr. Daniel Yoshor to our collaborative group. Dr. Yoshor is the new Chair of the Department of Neurosurgery at UPenn (see Dr. Yoshor's biosketch for more information) starting on June 1 of 2020 and will take over as the Chair of Neurosurgery on July 1, 2020. He will continue prior fruitful collaborations with Baylor College of Medicine, including those with Drs. Michael Beauchamp and Brett Foster. Dr. Yoshor will utilize the BlackRock system for research to develop cortical visual prostheses and to explore language, cognition and human behavior. These investigators also have extensive experience utilizing the Blackrock system at Baylor.

Because of these developments and this acute need, we are now requesting funding to purchase a 512-channel BlackRock NeuroPort Recording system and a companion 96-channel CereStim Stimulation system. In this proposal we present 8 major and 2 minor accomplished principal investigators who will aggressively utilize this equipment if granted. The equipment is also vital to our collaborative work at Penn, to define functional networks in normal human function and disease, and in our work to design and build new brain- computer interfaces, implantable devices to treat brain disorders, to improve intra- operative neurophysiology, and to push forward our research into therapeutic brain stimulation.

Accessible User Time (AUT): Currently we have between 35-40 implants in the Penn Epilepsy

Center per year, each lasting 1-2 weeks (average length of stay 10 days, average EEG monitoring per patient 240 hours, conservative estimate of total number of annual hours 8,400 hours). We expect that this number will grow dramatically with our new, larger EMU, but we use the current numbers as a conservative estimate of potential usage). Each of these patients will be a good candidate for the detailed major and minor user projects. Our estimate is that we will record a minimum of 35 patients per year, each continuously for a minimum of 7 days, with breaks for other studies, for a utilization of > 5,000 hours/year. Our Center for Neuroengineering and Therapeutics has ongoing support from the University of Pennsylvania School of Medicine that covers salaries for 5 staff who centralize IRB submissions for human intracranial physiology research, perform experiments on our patients for all investigators submitting research protocols for testing on our shared cloud-based data portal, www.IEEG.org, and assist with submitting Investigational Device Exemptions (IDEs) for new hardware and software to be tested in the EMU and operating room in our patients, including novel neuro recording and stimulation technologies. This investment is ongoing and exemplifies Penn's institutional commitment to our work and the collaborative research group that will utilize the requested equipment.

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Sharp – Justification of Need

Recent progress in structural biology and molecular biophysics (SBMB) has been rapid, both in terms of scientific advances and technology advances. This very quantitative area of biomedical science has traditionally had a large requirement for computing, and moreover has often driven the development of new algorithms and software. For some time, researchers in structural biology and molecular biophysics at the University of Pennsylvania have faced a growing computational bottleneck, which is now so acute that it can only be alleviated by a high end computing cluster specifically designed to support SBMB. Specifically, we require a targeted facility designed to leverage existing instrumentation and to assist research groups working in the areas of Cryo-Electron microscopy (Cryo-EM), Nuclear Magnetic Resonance (NMR), X-ray crystallography and scattering, single molecule biophysics, high resolution mass spectroscopy (MS), Hydrogen Exchange (HX) and macromolecular simulations. In these areas, we have 13 labs that include PI's on 26 different NIH funded projects with over $6,700,000 of NIH support per year. These research programs address important questions in diseases such as amyloidogenesis and neurodegenerative disorders, diseases of protein-misfolding, gene regulatory proteins and their aberrant regulation in cancer, defects in myosin leading to hypopigmentation and neurological disorders, identification and structural characterization of new protein targets for drugs and therapeutics. Of course, we are aware that many other areas of modern biomedical science require serious computational resources, such as database mining, bio-informatics, sequence searching and genome wide association studies. **The advent of highly distributed computing and cloud-based computing, and their positive impact on the areas just mentioned, should not obscure the fact that there are crucial differences between different types of computation, and that no one hardware solution is optimal for every computational task in modern biomedical research.**

To delineate the specific computational problems faced by structural biology and molecular biophysics, and demonstrate why they are best solved by dedicated hardware, we frame this in terms of 'dense' computational tasks.

A.1 Dense Computing: An essential component of Structural Biology and Molecular Biophysics

Defined by one or more of the following features:

1) High algorithmic connectivity: Every sub-component of the computation must exchange data frequently with multiple other components; one cannot partition the task into semi-autonomous parts.

2) Frequent access to, or production of, petabyte scale data during the computations.

3) Supra-linear scaling with size of system, typically resulting from 3-dimensional or higher dependence on the size of the system being studied, fineness of sampling, resolution of the experimental data, etc.

The following list illustrates the major forms in which dense computational tasks arise in SBMB:

Table A1. Dense Computing in Structural Biology and Molecular Biophysics (SBMB)

Computational Area	General class of Algorithms
Cryo-EM 3D reconstruction	Optimization/Sampling
NMR data processing	Spectral Transform/Spectral Analysis
Molecular Simulations	Integration, Sampling
Small Angle Scattering analysis	Sampling/Optimization
Xray Crystallography	Spectral Transform/Optimization
Mass Spec data analysis	Optimization
Quantum Mechanics	Integration, Spectral transforms

Because of their algorithmically dense nature, these tasks do not scale well to very large numbers of compute nodes, and they are not optimally addressed by highly distributed computing and cloud-based computing. Given the truly massive amounts of data (multi-terabyte to peta-byte in size) involved in some of these tasks, especially Cryo-EM, there are also bottlenecks in using off site resources such as national super-computing facilities due to multi-day transfer times arising from 1-10GB network speed restrictions outside the facility.

The third feature of 'dense computing' – supra-linear scaling with size of system – also explains why the very success of SBMB at the University of Pennsylvania has lead to the current computational bottleneck: In general, people are studying larger and larger multi-molecular complexes, molecular assemblies and molecular machines, using higher and higher resolution methods.

- a) Higher resolution Cryo-EM: The size of the images goes up as the square of the resolution. b) The ability to prepare and then image larger bio-molecular complexes: the 3D reconstruction computations scale at least as the third power of complex size.
- X-ray crystallography: A similar 3rd power scaling applies as X-ray crystallographic refinement and molecular simulations are applied to larger multi-molecular assemblies.
- Higher resolution, multidimensional NMR: Going from 2D spectra to 3D spectra, and now to 4D spectra means moving to the fourth power of resolution. New methods of non-uniform sampling and spectral analysis use larger computational resources.
- Mass Spectrometry: As modern Mass Spectrometry instruments are combined with techniques like hydrogen exchange, stable isotope labeling (SILAC), and more efficient fragmentation of larger and larger molecules, the number of mass fragments to be isolated, identified and characterized increases combinatorially.
- Quantum mechanics: As new and often larger optical imaging probes are developed, incorporating novel fluorescence properties, or two-photon probes are developed, expensive quantum mechanical calculations of larger numbers of atom and/or excited state properties are needed. These scale at least with the third power of size.

In each case, the larger computational task cannot simply be partitioned among a larger set of compute nodes without incurring a large inter-node communication penalty: For example with any task involving spectral

transforms (e.g. fast Fourier Transforms), each point of the transform is a function of every original datum. In any task involving 3-dimensional structures, the data representing the dense set of atom-atom interactions must be transmitted to each part of the computation.

A.2. Current computing resources.

The current Penn High Performance Computing (HPC) resources are housed and administered by the Penn Medicine Academic Computing Services (PMACS). HPC consists of about 144 16-core CPU-based nodes, which are primarily suited for highly distributable tasks such as database searching and mining, sequence searching and biomedical-informatics data mining. The facility has three serious limitations for types of computation required by SBMB. First, the dense computing algorithms used by SBMB applications have relatively low throughput and don't scale well on the typical number of cores available from PMACS at any time. This assertion is supported by pilot test calculations on the PMACS HPC facility using both Cryo-EM data analysis by Professors Marmorstein and Ostap and macromolecular simulations by Professor Paul Axelsen (see Project Section C). Second, there are logistical problems in transferring the 100Tb to Petabyte scale data to and from the PMACS facility: It is off site, and even with fast networking, data transfer can take many hours and sometimes days. The PMACS HPC site is a secured facility due to the presence of patient data, which places barriers to the transfer of data by the most efficient means - removable external hard drives. Finally, the resource has about 2 petabytes of online storage, of which about 95% is already used. Cryo-EM alone, using either the current University of Pennsylvania Microscopy core or the offsite EM facilities in use by University of Pennsylvania labs, can generate petabyte scale data on a single high resolution structure project. Clearly, from both computing complexity and data size considerations, an additional on-site computer cluster, designed from the outset for Structural Biology and Molecular Biophysics, is required.

A.3. Hardware

The proposed hardware will make heavy use of state of the art graphics processing unit (GPU) hardware, with an emphasis on shared memory and high through-put. In outline, the proposed equipment consists of 5 nodes with dual 10-core CPU's plus 6 Tesla K80 GPU nodes. GPU-optimized software required for the most computationally intensive Structural Biology and Molecular Biophysics applications has already been developed. NVIDIA, maker of the proposed GPU units, also provides software and technical support at no extra charge to implement scientific solutions on their hardware. Given this available software, GPU-based computation now represents the best performance/price ratio in high end computing. A white paper from the Intel Throughput Computing Group quotes a factor of about 2.5 in favor of GPU's (pcl.intel-research.net/publications/isca319-lee.pdf). The proposed hardware will be housed in the same location as the Structural Biology core instrumentation, where it will physically replace an obsolete, 12-year old SGI Altix cluster. Due to major advances in computer hardware, the requested equipment occupies about the same footprint, and has about the same power and cooling requirements as the cluster it will replace. Based on experience with our two previous clusters, we budget about 1 day per month downtime for system maintenance, operating system software upgrades (when the cluster will be unavailable to users). Other system maintenance, software installations and upgrades, job scheduling and other tasks needed to administer the cluster can be performed while the cluster is in use. Then, aside from any downtime due to hardware replacements, available user time is essentially 24hrs per day, 7 days a week, amounting to more than 8,000hrs of available user time (AUT) per year.

A.4. Software

Effective use of the hardware requires the availability of software optimized for that hardware. Such software exists for all the major computational tasks described in Table A1. The following software will be installed on

the hardware. Website addresses are included solely to document the availability of the appropriate software for the requested hardware.

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Instructions for Technical Expertise (3 pages)*:* Describe the technical expertise of individuals who will set up and run the instrument. Specify who will ensure that the instrument is safely operated and appropriately maintained. State who will train new users. If the instrument requires complex sample preparation or consultation for experimental designs, describe the expert individuals who will serve in that capacity. Address technical support for data collection, management, and analysis.

- **Section Guidelines:** As adapted from the ASCB, this section should include the following:
    1. The day-to-day use, oversight, and maintenance of the instrument including a discussion of the expertise of the PI, user group, and staff.
    2. One or two paragraphs discussing the technical/scientific advisory committee. Mention advisors and consultant(s) who will advise on experimental design, use, and application.
    3. Data management and infrastructure that supports use of the instrument.
    4. A brief summary of biosafety/biohazard protocols.

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Goldman – Technical Expertise

The director of the facility and PI, Dr. Yale E. Goldman, MD, PhD, will oversee operation. He has had a long-term involvement in single molecule biophysics, particularly directed toward molecular motors and protein synthesis. He has made use of advanced instrumentation and developed and applied novel biophysical techniques to these studies, including laser photolysis of caged compounds, nanometer tracking of fluorescent probes, polarized total internal fluorescence microscopy for structural dynamics, high speed optical traps, and "parallax view" 3D tracking. Dr. Goldman was Director of the Pennsylvania Muscle Institute, a mid-sized research consortium in the Philadelphia area, for ~20 years and is currently Co-Director of the Nano/Bio Interface Center, Penn's nanotechnology institute. He has had leadership positions in the scientific community, such as Presidency of the Biophysical Society, Associate Distributing Editor for Biophysical Journal, and Chairman of a Gordon Research Conference. Thus he has considerable administrative experience managing scientific research programs including intensive multi-investigator efforts and advanced instrumentation.

The Co-PI and Director of post-acquisition signal processing, Dr. Paul H. Axelsen, MD has extensive published experience in both fluorescence spectroscopy and molecular dynamics simulation, contributed code for parallel vector processors in early versions of CHARMm and pioneered some of the original studies that tested the validity of these simulations with fluorescence, NMR, X-ray crystallography, thermodynamic measurements and, most recently, two-dimensional infrared spectroscopy. He has recently competitively renewed NIH-R01 support for "Structure Determination by Vibrational Spectroscopy" which has a large computational chemistry component. Dr. Axelsen has had many leadership positions in the scientific community, having organized several large meetings and serving on the Editorial Board of Biophysical Journal. He is currently Treasurer of the Biophysical Society and as a member of the Biophysics of Neural Systems study section. Thus he also has the administrative experience and scientific perspective to contribute to operating the present initiative.

The Facility will be operated by Serapion (Ionas) Pyrpassopoulos, Ph.D., an expert in protein purification, calorimetry, membrane biophysics, and single-molecule biophysics. Dr. Pyrpassopoulos utilized and developed the optical-trap-based, single-molecule, membrane-adhesion assay and planar and spherical supported lipid bilayer technologies used in the Ostap Laboratory. He is also an expert in fluorescence microscopy as-says, and he will be responsible for collecting the preliminary MFD-FRET data on IHF-DNA in this proposal. Dr. Pyrpassopoulos co-directs the PMI's, P01-supported "Advanced Optical Microscopy and Instrumentation Core." In this duty, Dr. Pyrpassopoulos maintains advanced imaging and force-spectroscopy equipment, trains users, schedules usage, and manages scheduling conflicts, and is thus well suited to operate and maintain the currently requested instrumentation and train users. Dr. Claus Seidel in Dusseldorf Germany, has offered to have Ionas visit his laboratory to obtain further training in MFD-FRET experiments and additional analytical software that Dr. Seidel has developed. Ionas would bring back these advanced methods and programs from Düsseldorf to our facility. This is a huge opportunity to enhance UPenn and broader United States expertise on these powerful methods.

Dr. Elizabeth Rhoades, PhD, is a consulting faculty member in the Department of Chemistry and member of the Internal Advisory Board. Dr. Rhoades has used fluorescence correlation spectroscopy and single molecule FRET in extensive studies of microtubule binding and intrinsically disordered proteins. She has developed instrumentation similar to, but less capable than, the MFD-FRET technology requested here and is an expert in collecting, analyzing and interpreting the FRET signals to be obtained. Thus her advice to users about experimental design and interpretation will be highly valuable.

The Technical Facility Manager will be responsible for the day-today operation of the facility, including all aspects of project management including probe placement, fluorescent labeling methods and protocols, MFD-FRET experiments and follow-up after experiments. Dr. Pyrpassopoulos will report directly to the Director for this role. An Internal Advisory Committee (IAC) has been organized comprising Dr. Rhoades, Dr. Louis Soslowsky, Associate Dean for Core Facilities, Dr. Sergei Vinogradov, a probe and optics expert in the Dept. of Biochemistry and Molecular Biophysics, and Drs. Axelsen and Goldman, ex-officio. Dr. Kevin Foskett, Chair-man of Physiology, will serve on and Chair the IAC.

B.1. Key personnel

Yale E. Goldman, MD, PhD, PI and Scientific Director
Dr. Paul H. Axelsen, MD, Co-PI and Data post-processing Director
Dr. Serapion (Ionas) Pyrpassopoulos, PhD, Technical Facility Manager

The acquisition of a single molecule fluorescence multi-parameter fluorescence detection resonance energy transfer (MFD-FRET) microscope fulfills a clear currently unmet need for such studies at this institution. To

exemplify these needs there are 6 major users who represent 3 different departments within the School of Medicine. These users will account for 56% of the usage. Furthermore, there are 9 minor users who will account for 20% of the usage. The goal is to support this group of 12 NIH funded investigators (accounting for 76% of the usage) along with additional users, with preference to the major users. The remaining instrument time will be available for new users, experimental development, and maintenance.

B.2. Major Users of this Equipment/Facility (These tables have been expanded and modified, does it sufficiently answer the referee criticism of documenting need for accessories?)

Last name	First name	Department	NIH grant numbers	Est. % of Use	FRET	PIE	Aniso-tropy	Quad detec-tors	Pola-rizers	Wave-lengths (nm)
Major Users										
Goldman	Yale E.	Physiology	P01-GM087253 R01-GM086352 R01-GM080376	12	X	X	X	X	X	440 532 640
Axelsen	Paul	Pharmacology	R01-GM076201 R01-NS074178	8	X		X	X	X	440

'X' notations indicate usage by each investigator of the main techniques supplied by the requested instrument.

B.3. Minor Users of this Equipment/Facility

B.3. Minor Users of this Equipment/Facility

Last name	First name	Department	NIH grant numbers	Est. % of Use	FRET	PIE	Aniso-tropy	Quad detec-tors	Pola-rizers	Wave-lengths (nm)
Minor Users										
Deutsch	Carol	Physiology	R01-GM052302	3	X		X	X	X	532 640
Hoshi	Toshinori	Physiology	R01-GM57654	3	X	X				440

X' notations indicate usage by each investigator of the main techniques supplied by the requested instrument.

B.4: Biosafety

None of the proposed projects require biosafety protocols for the use of this instrument. If there are re-quests to expand the coverage to include biosafety protocols our Scientific Advisory Committee will discuss this issue.

B.5: Conduct of Experiments

Users of the MFD-FRET instrumentation will design their experiments in consultation with the facility manager (Dr. Pyrpassopoulos) and the computational component for the purpose of (a) defining the model to be studied, (b) planning where donor and acceptor labels may be feasibly and most informatively placed, and (c) deciding which donor and acceptor label pairs to use. For many of the experiments foreseen, which are primarily occupancy, ligand association and dynamics will be able to analyze data using user friendly, publically available software, e.g. ref. 7 above, supplied and taught by facility staff. For projects requiring more precise distance values requiring molecular dynamics (MD) modeling of the probe motions, we will determine at the inception of a project, whether their experience with MD simulations is sufficient for modeling their system, or will match them with one of the many labs on campus that routinely do these simulations (e.g. Axelsen, Sharp, Radhakrishnan, Dunbrack, Saven, Rappe, or most of the crystallography and NMR labs).

It is important at this initial stage to consider whether a reliable model may be generated, so that one may forecast whether the data to be collected will answer the questions posed. Ultimately, our ability to interpret the data will depend on the quality of the model. It should be noted that most of the projects described in this application by potential users of the instrumentation involve single proteins, well-defined protein-protein inter-actions, or protein-nucleic acid interactions, where there are substantial amounts of high precision structural information available before any FRET studies will be performed. Each investigator is proposing FRET studies on systems they have already been working on for many years, and in most cases, crystallographic or NMR structures are available. Therefore, each of the projects described will start with a high quality model available readily available and familiar to the user.

B.5.a: Site-specific fluorescent labeling.

To report distances and structural changes in macromolecules, FRET experiments require introducing suitable pairs of fluorophores at defined sites. Several approaches can be used, depending on the requirements of the particular experiment. For proteins, the oldest method, still widely-applicable, is direct chemical labeling of reactive side-chains with small organic fluorescent reagents. Additional methods include enzymatic coupling of small fluorophores, and fusion with fluorescent proteins or with self-labeling enzymes. A few labs have used the incorporation of unnatural amino acids to expand the set of options for specific chemical labeling. Where the desired measurement includes sites on substrate molecules, nucleic acids, or small peptides bound to proteins, fluorescent groups can be introduced by direct synthesis. Different experimental systems are likely to require the opportunistic use of different strategies. Facility staff and collaborative laboratories at Penn have extensive experience with the full range of protein conjugation and expression strategies. We will assist and instruct users who are less experienced or match them with the extensive and collegial available expertise.

B.5.a.i. Labeling at cysteine residues. The high reactivity of thiols with alkylating agents allows cysteine residues to be labeled under mild conditions, with little or no labeling of other side-chains. A large variety of cysteine-reactive dyes is available, and a further advantage is that, in contrast with many fusion proteins, engineered cysteines need not be restricted to the N- or C-terminus of the protein. The simplest case would be a protein with no intrinsic reactive cysteine residues, where cysteine can be substituted for uncharged, polar residues (e.g. serine, threonine) at locations appropriate for the distances to be measured. Simultaneous or sequential labeling with donor and acceptor fluorophores would then result in a mix of labeled proteins, at best half of them labeled with one donor and one acceptor fluorophore. A critical advantage of single-molecule FRET versus ensemble measurement is that the signals from such double-labeled molecules can be distinguished from those of molecules labeled with two donors or two acceptors.

For this strategy to be feasible, it is first necessary to determine whether any of the protein's intrinsic cysteine residues (if present) are reactive. In past studies on myosin V and myosin VI, which each contain more than 10 cysteine residues (depending on where the construct is truncated), it was necessary to identify which intrinsic cysteines were reactive. The isolation of labeled tryptic peptides, from a digest containing dozens of components, was much facilitated by affinity chromatography using immobilized β-cyclodextrin6, allowing for subsequent identification by ESI-MS. The resulting data allowed the design of 'cys-lite' constructs. While the most reactive intrinsic cysteines were mutated to non-reactive residues, others were essential to maintain native structure and activity, and low levels of labeling were still observed at some of these remaining cysteines. This background labeling was much reduced by using the reactive dye as an inclusion complex with soluble β-cyclodextrin: intrinsic cysteines are generally somewhat buried, in moderately- to highly-hydrophobic environments, while engineered cysteines are usually placed on solvent-exposed surfaces.

Encapsulating the dye molecule in a more bulky, hydrophilic complex thus further restricts its access to intrinsic cysteines relative to those introduced on the surface.

B.5.a.ii. Enzyme-mediated labeling. Alternative labeling strategies may be necessary, particularly where an intrinsic cysteine residue is highly reactive and cannot be mutated while maintaining the protein's native structure and function. Both mammalian and bacterial transglutaminase have been used to couple fluorophores and other small molecules with reactive amino groups to glutamine side-chains. Since glutamine occurs more commonly than cysteine, and the reactivity of any particular glutamine residue is not readily predictable, transglutaminase-catalyzed labeling is less generally applicable than labeling at cysteine; however, the availability of the enzyme (particularly bacterial transglutaminase) and suitable dye substrates makes it worth investigating in cases where labeling at cysteine is not sufficient. Transglutaminase-catalyzed labeling shares the advantage of not being restricted to sites at the N- or C-terminus of the protein: e.g., Q41 of actin, which is located on a flexible loop, has been labeled with high occupancy and specificity using both mammalian and bacterial transglutaminase3,8. Glutamine-containing sequence tags that are particularly avid substrates for labeling have also been reported1,9. So far, these tags have been inserted only at the N- and C-termini of expressed proteins, but the example of actin Q41 suggests that insertion and labeling at internal sites may also be feasible.

A lipoic acid ligase construct has been used to couple a small fluorophore to a 13-residue sequence tag2. This system, which is not yet commercially available, is so far limited to the N- or C-terminus, but has the advantage of high specificity and avoids adding the bulk of a protein fusion partner.
B.5.a.iii. Fusion partners. Fluorescent proteins, such as GFP and its spectral variants, and enzymes that self-label with fluorescent substrates (e.g. HaloTag, SnapTag), have been most widely used for intracellular imaging, but have also been used for intramolecular FRET measurements4,7. This strategy has the obvious advantage of eliminating background labeling at other sites, but the choice of labeled sites is then limited to the N- or C-terminus of the protein. Both the added size of the fluorescent partner, and the usual need for a flexible linker of significant length to make it compatible with the protein being studied, limit the applicability of this strategy.

B.5.a.iv. Labeling of unnatural amino acids. The biosynthetic incorporation of unnatural amino acids (Uaas) into expressed proteins was pioneered most notably by the Schultz group10, and has been exploited by an increasing number of labs. Milles et al.5 recently reported detailed methods for the incorporation of several different Uaa's in bacterially-expressed constructs, and their subsequent fluorescent labeling by click-chemistry. Like cysteine, Uaas can be incorporated at any desired position in a protein, allowing the sites to be selected so as to provide the most useful information. Plasmid pEVOL-pAzF, incorporating the suppressor-tRNA and synthe-tase for p-azidophenylalanine, was developed by the Schultz lab and is available from AddGene. Both pAzPhe and suitable reactive dyes are commercially available. Without discounting the complexity of implementing this method in a new lab, it offers an attractive alternative where fluorophores must be introduced far from the N- and C-termini, and where labeling at cysteine or glutamine is not satisfactory.

A great range of strategies and expertise are available from facility staff and within the university.

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B5.b: MFD-FRET Measurements

Measurements for a typical user will be carried out by loading the experimental and control samples into covered eight-well plates pre-treated with polylysine-conjugated polyethylene glycol as a passivating agent. From earlier single molecule studies we have extensive experience with methods to avoid non-specific contact of proteins, nucleic acids, nucleotides and other small molecules. In single molecule burst-mode MFD-FRET experiments, sample fluorophore concentrations are typically set at 10 – 50 pN to establish single-transit non-colliding recordings. Total photon count thresholds for including events in the subsequent analysis is provided by the PicoQuant software. At 5 µW input laser power, ~1,000 photon bursts over 100 counts per event are typically recorded in 5 minutes of survey acquisition and for production experimental runs, 6,000 – 10,000 qualified events can be accumulated in 30 min. The commercial software takes into account the majority of analysis and correction tasks and readily provides plots and secondary data (Fig. 6). The most common equation for FRET efficiency is EFRET = Iac / (Iac + γIdc), where Iac = (Ia – ba) – β(Id – bd) and Idc = (Id – bd) + β(Id – bd), ba and bd are background acceptor and donor counts, γ accounts for differential collection efficiency of the donor and acceptor channels (including the detection filters and acceptor QYs), and β describes leakage of donor fluorescence into the acceptor detector channel. The software takes these factors into account and provides convenient methods to measure the relevant parameters. One of the advantages of purchasing a commercial instrument is to make these operations user-friendly and they will require only a limited amount of training. Similarly, procedures for obtaining anisotropy, time resolved anisotropy decay, dynamic kinetics and stoichiometries are pro-vided in the user menus. Data will be stored temporarily on the operational computer and then transferred via the campus network to the local analysis workstations and then to users' laboratories. As mentioned, Dr. Pyrpassopoulos has been invited by Dr. Claus Seidel to visit Düsseldorf for high-level training in the most advanced software for more specialized analysis methods. Please see Dr. Seidel's letter of support.

B.5.c: Post-processing, computational methods and interpretation

For example, our molecular dynamics simulations of elongation factor TU (EF-Tu) with Cy3 attached to residue 33, and Cy5 attached to residue 351 (Fig. 7), suggest that the inter-fluorophore distance should increase from 3.9 with bound GDP to 6.4 nm with bound GTP (Fig. 8 in Project C.1.a). If $\kappa = 2/3$ in both cases, then = 5.0 and should decrease. However, the simulations also suggest that κ will increase from ~0.1 to ~2.2. Therefore, should increase despite the greater interfluorophore distance, instead of misleadingly suggesting that it decreases. The possibility that FRET measurements may be so wildly misinterpreted underscores the need for precise characterization of κ.

It is widely recognized that a single value of κ must represent a distribution of orientational possibilities. Many approaches to the estimation of these distributions have been described, including rigid-body rotation schemes and molecular dynamics simulation.5-16 The most recent and advanced approach is the FRET positioning and screening (FPS) system,17 a hybrid approach that employs molecular dynamics simulation to determine the distributions of donor and acceptor orientations, and then fixes these "accessible volumes" to docking macromolecules that are then manipulated as rigid bodies. An ensemble of possible structures is generated at random, and steric clashes that arise either eliminated or relaxed by various functions, e.g. harmonic potentials. Finally, the results are filtered with respect to their agreement with the FRET measurement. FPS is available as a "toolkit" for free download from the authors and will be available for routine use in the facility.

Thus, molecular dynamics simulation is already a significant and important component of modern FRET interpretation. FPS with simulations has been applied to a DNA-protein complex with impressive results,17 but molecular dynamics simulation is only used at an early stage of FPS. Whether or not this approach is sufficiently robust for application other systems has yet to be tested. The rationale offered in ref. 17 for not modeling dye behavior with simulations during the later stage filtering steps is that they "are time consuming", whereas treating dye behavior as a fixed accessible volume is fast enough for filtering data and initial conclusions.

There is a large class of potential FRET applications for which a full-scale molecular dynamics simulation is not only feasible, but highly advantageous (by "full scale" we mean all-atom fully-solvated simulations in periodic boundaries). These applications include those in which the donor and acceptor are both attached to the same macromolecule for the purpose of probing its function or the effects of ligands. EF-Tu mentioned above is an example: it is well within the computational capability of many labs to conduct full-scale simulations of EF-Tu on a timescale that is many multiples of the fluorescence lifetime, and this approach offers several advantages over FPS. One is that the effects of solvent may be explicitly included. We should expect solvent to have highly significant effects on the distribution of dye orientations – limiting some otherwise "accessible" volumes, while favoring others. A second advantage is that more appropriate averaging schemes may be applied for donor-acceptor orientations; instead of the average donor orientation being matched to average acceptor orientation, simulations make it possible to average the instantaneous relative orientation of donor and acceptor. An appropriate averaging scheme (weighted by r6) is important because of the subtle but real distance dependence of κ. This dependence means that distance contributes to both the numerator and denominator of the $/$ term to a degree that could have significant effects on, but which have not yet been studied with sufficient precision.

A third advantage is that correlated donor-acceptor motions are possible, and simulations permit the effect of donor fluorescence lifetime to be treated explicitly by averaging the orientation of the donor relative to that of the acceptor at a later time point. A fourth advantage is that motions of the fluorophore attachment point may be simply and unambiguously separated from motions of the fluorophore. Fundamentally, motions of the underlying macromolecule are of interest, rather than those of the fluorophores.

A final – and perhaps the most important – advantage of full-scale molecular dynamics simulations is that they ultimately yield a complete model of the system being interrogated. Not only does such a model provide insight into parts of the molecule not tagged with fluorophores, but it provides a framework for establishing that different donor-acceptor combinations, or different donor and acceptor locations, yield consistent results.

The obvious questions arising at this point are: if simulations are accurate, then why is experimental data needed? And, if they are not accurate, how can they inform our interpretation of the experimental data? The answer to both questions is that both simulation and FRET experiments are subject to errors. When combined to yield mutually consistent, synergistic results, however, their reliability is high and their value is far greater than the sum of their individual contributions because full-scale simulations offer insight into changes in orientation, anisotropy, and dynamics in the form of a distance distribution, and each of these characteristics may be compared to experimental data from an MFD-FRET experiment.

In most cases, the preparation for data analysis will not begin until it is clear that reproducible data is forth-coming from a system, and that it is sufficient in quantity to analyze. From planning consultations, we will have inventoried the structural information available about a system, and determined the kind of computational model needed for analysis. In many cases involving systems with defined tertiary structure or single proteins, we anticipate that these plans will call for full-scale molecular dynamics simulations. For multi-subunit systems where the macromolecular interactions are not defined, a search and screen approach such as FPS will be more appropriate. It should be noted that the authors of the FPS system have made their software freely avail-able online and that Dr. Pyrpassopoulos will have the opportunity to learn its potential and limitations first-hand.

The preparation of a full-scale molecular dynamics simulation takes experience, time, and skill to make the simulation well-behaved and well-equilibrated. We expect that relatively few users of this facility will have suit-able experience in molecular dynamics simulation. However, there are many labs on the Penn campus and around the world with suitable experience in molecular dynamics simulation (including some of the potential collaborators listed above). Moreover, the co-director (Axelsen) and personnel in his lab will be available for consultation. Therefore, access to MFD-FRET instrumentation is the research bottleneck, and access to computational expertise and resources is readily available.

The length scale over which most FRET experiments will be conducted (1-10 nm) informs us that the size of the molecular dynamics simulations needed in most cases is readily feasible, even if only a "region of interest" must be selected from a larger system. By "feasible", we mean that a system of sufficient size may be equilibrated and run for several multiples of the fluorescence lifetime (e.g. tens-of-nanoseconds). One might expect a reasonably experienced postdoctoral fellow to prepare a set of systems with variously positioned do-nor-acceptor pairs in 1-2 days, and 1-2 weeks of wall-clock time on one of Penn's available high-performance computer clusters to equilibrate each of them (the actual times for our EF-Tu simulations in Fig. 7).

Given an equilibrated system, the path to a conclusion will depend on the type of data collected, the nature of the system, and the questions being asked. Extending the simulation to create a "production" trajectory is straightforward. Experimental observables measured in a FRET experiment such as relative orientation (from polarized detection) and anisotropy decay are readily derived from a simulation, as is κ for distance determination. Therefore, the first task in post-analysis is to determine whether the simulations are consistent with the MFD-FRET results, including the measured anisotropy. If not, then the collaborating investigators (experimentalist and simulator) must decide what must be done to the simulations to explain the results. Here, the power of MFD-FRET is appreciated, because characteristics of the system in addition to distance have been measured (i.e. anisotropy and polarization experiments), and this data both informs and constrains the simulation. Simulations in which bias potentials are needed to fit the data are only slightly more complex than unbiased simulations, but the greater difficulty is deciding how to alter the simulation. Simple options include titrating side chain charges (esp. His) and changing the nature or position of counterions. More complex options include restraining potentials distributed over a portion of the structure. Ultimately, the approach to reconciling experiment with simulation is system-specific, but often extremely valuable as it requires the investigator to question the assumptions about structure made at the inception of the experiment. The facilities, expertise and interest in this aspect of the research abound at the University of Pennsylvania, making the after-measurement computing task a natural and useful procedure. Help by experts at the MFD-FRET facility and in the local environment is readily at hand.

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Gupta – 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.

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Litt – Technical Expertise

Instrument Expertise:

Users have both instrument specific and domain relevant knowledge to ensure immediate and expert useof the proposed BlackRock electrophysiological system, as noted above. This is both from our experiencewith the DARPA RAM project, and the experience of Dr. Yoshor's group at Baylor College of Medicine before his relocation to Penn in 2020. In addition to our RAM experience, PI Yoshor and his team have specifically utilized the BlackRock system for invasive human electrophysiology since 2009. This use hasevolved over the years through improved iterations of the BlackRock system, including a recent upgrade(BCM) to the current NeuroPort system requested. PI Yoshor has employed the NeuroPort system and associated CereStim device for performing invasive recording and stimulation experiments, which have required expert device usage, including the development of custom software and hardware interfacing. Reflective of this expertise, PI Yoshor and his team were the first BlackRock users to employ and pilot a new

connectivity setup for interfacing the NeuroPort/Cerestim system with a common EMU hardware formore streamlined usage that minimizes clinical disruption. This process provided a detailed understanding of both the Neuroport/Cerestim systems, along with expertise in troubleshooting soft/hardware functionality. Scientifically, this expertise is born out in a host of high impact publications, all based of data obtained via the BlackRock system.

Similarly, the Penn Epilepsy Center faculty, PIs Litt, Davis, Lucas and Richardson, and their teamalso have extensive experience with the BlackRock Neuroport and Cerestim system for invasive studiesof the human brain. This expertise also involves detailed work in custom device usage for simultaneousrecording and stimulation experiments. Indeed, the BlackRock Neuroport system was employed in a priormulti-center study of human memory (Restoring Active Memory, RAM; DARPA) requiring detailed knowledge of the system. Data generated through this project as well as other studies utilizing the sameBlackRock system, and under Dr. Litt's prior S10, utilizing the Neuralynx system have resulted in a host of impactful findings (see publications embedded in the Research Projects below). These findings includea large body of findings that have helped shape our understanding of high frequency oscillations in epileptic networks, using network models to plan "virtual cortical resection," and publications developing new high-resolution hardware to map brain networks in animal models and humans.

In addition to several primary users with intimate expertise in the BlackRock Neuroport/Cerestim system, multiple users are world experts in utilizing electrophysiological hardware for performing invasive neurophysiological experiments in humans and non-human primates for both clinical and basic science investigation. Physician scientists Litt and Daivs are both world experts in the electrophysiological studyof epilepsy and seizure dynamics in the human brain together with collaborator Bassett. Drs. Lucas and Richardson have extensive experience developing new devices to map somatosensation in human brain,Dr. Gottfried is a world expert in circuits related to olfaction, memory and cognition, Dr. Cohen in auditory cortex, and Drs. Platt and Gold in reward, behavior and decision making. These are just a few examplesof the expertise available at Penn to utilize the requested equipment. All of these investigators have experience in high resolution recording and electrophysiology in humans and animals, including non-human primates, that they will port to human experiments in the EMU using the Blackrock system.

Together, the user base at UPenn has the technical expertise specific to the requested BlackRock system in addition to detailed domain expertise in human and non-human primate electrophysiological systems, as evidenced by users published work in multiple fields of clinical and basic science. This expertise will ensure that instrument usage can occur immediately and at a sophisticated level. In addition, this broad expertise will ensure safe and optimal operation of the instrument, along with its maintenance. User expertise will therefore serve as the primary means for training new users, which willbe supported by specific training workshops as detail below.

Instrument Operation Safety:

Overall the instrument presents limited safety concerns, however several procedures are in place to ensure its safe use. During any subject recording involving the instrument, as with past use of our Neuralynx and DARPA RAM Blackrock systems, there will be multiple team members present during device operation, each with specific roles. While one team member will work with the subject to describe and guide experiments, a minimum of two other team members will be responsible for instrument operation – focused on managing data recording and monitoring ongoing signal quality. As recording is passive, there's a limited safety risk to the subject and user. As noted below, new users will shadow

expert users as part of the instrument training. Additional safety measures will be in place for use of the Cerestim components, used for electrical brain stimulation. During use of the Cerestim, there will be a clinical team member in the EMU to monitor experiments, reading the IEEG continuously to observe for after-discharges or disruption of normal brain function. Operation of the Cerestim device will only be performed by specifically training expert users. The UPenn team has extensive experience with invasive electrical brain stimulation and detailed understanding of its safe parameter ranges. All stimulations will be kept strictly below nationally recognized limits of safe current density delivery through intracranial electrodes. In addition, it is our policy that senior EEG technologists are present on the EMU at all times, one of which is deployed to every high-resolution recording and stimulation patient. This senior technologist will trouble shoot and observe equipment, patient and operation during every stimulation experiment, to insure safe equipment operation and patient safety. We have found over the years that these protocols have guaranteed smooth, safe experiments, and we have reported no severe adverse events on our EMU during equipment operation for any of our experimental protocols.

Beyond personnel safety considerations, several logistic and hardware solutions will be employed. Firstly, to ensure no damage to the instrument, a fixed 'power on' protocol will be in place, aspart of the experimental testing log. This includes a fixed sequence of device power on and connection/cable checks. Secondly, isolating power sources with surge protection will be used for the instrument to further protect from device and user/subject damage or injury. To maintain safe instrumentuse, device issues will be part of weekly meetings for primary users to raise any challenges with instrument operation and plans to solve them. As Blackrock use will be in parallel to standard equipment operation, careful attention to hospital regulations requiring a single ground for all devices connected to a patient will be strictly adhered to, in addition to frequent measurements of any leakage current from clinical devices and verification of device isolation at regular intervals, as defined by hospital clinical engineering, will be strictly adhered to. We have found that these protocols have guaranteed safe, efficient operation of device during over a decade of this type of research at Penn.

Instrument Training:

New users of the instrument will go through a structured onboarding process, which involves conceptualand hands on training via key patient/participant testing personnel and faculty. Each new user will be provided with documentation of the instrument highlighting key hardware components and software. Next, new users will go through hands on training via first shadowing expert users during experimental recordings, followed by hardware piloting during non-experimental periods. Shadowing will be chiefly provided by post-doctoral fellows and dedicated technicians and technologists responsible for conductingexperiments. After this training, any new user will conduct experiments under the supervision of an expert user until they show clear independence to use the instrument accurately and safely. All experiments using the instrument will involve multiple team members, helping to ensure safety oversite.

In addition to onsite instrument training provided by UPenn users, new users will also attend a hands-on workshop held by BlackRock microsystems multiple times a year to support device training. New users will specifically attend the multiday workshop focused on using the Neuroport/Cerestim system for human subject's research. PI Yoshor has previously had trainees attend this workshop, findingit particularly helpful.

Instrument Support:

Data collection: As detailed above, all experiments involving the instrument will include a key set of personnel for working with the subject and equipment. Our EMU research team already has dedicated personnel for running and coordinating experiments utilizing the instrument. Each user will have the support of this team to ensure safe and successful testing.

Data management: The BlackRock system will generate large amounts of multi-channel electrophysiological data. Initially, recorded data is stored locally during experiments. During testing session breaks or at the conclusion of testing for each day the data will be securely transferred to our server in the CNT for storage, and then to our cloud based portal, www.ieeg.org, for long term analysis.All testing will follow a detailed testing log to help document experiments. Data logging and transfer will be performed by the EMU research team and the CNT data management team, under the direction of Jacqueline Boccanfuso. Data stored on ieeg.org will be accessible to users for subsequent analysis. The storage infrastructure is supported by dedicated staff through the CNT. Data storage and archiving will follow predefined file structures and naming conventions to ensure long term accurate usage of data, supporting improved means of data sharing.

Data analysis: All users have extensive experience in the analysis of the data generated by the Neuroport system. Each user will have access to their generated data via the ieeg.org storage system. Subsequentlyeach user will conduct project specific analysis with relevant in-house software. The team has extensive experience in the analysis of electrophysiological data, along with multiple established pipelines for data processing. This expertise and extant software will provide a rich support for any new user to explore their data. Our research group maintains a shared GitHub infrastructure for analysis software and a group of tools and APIs in MATLAB and Python that are available for data analysis on ieeg.org. This infrastructure communicates directly with a variety of tools for analyzing time series and imaging data, including the FlyWheel system for image processing, using by many of our Penn collaborators, as well as internal and external resources developed by other investigators. ieeg.org is currently used by ~4,600 investigators worldwide for data sharing and analysis.

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Sharp – Technical Expertise

B.1. Housing and maintenance of the hardware

The equipment will be housed in the Structural Biology facility of the Biochemistry and Biophysics Department in the same room as the X-ray core facility and next door to the Cryo-EM core and NMR facilities. The room is already equipped with adequate 110V and 220V power, as well as adequate cooling. The requested hardware occupies a half-rack and will physically replace an obsolete SGI Altix cluster. From a housing, power, and maintenance perspective, the demand from the new equipment is little different from the equipment it will replace.

B.2. System maintenance and Software management.

The PI, Dr. Sharp, will be primarily responsible for system administration and software installation and upgrades on the cluster. Dr. Sharp previously administered a cluster of 6 Silicon Graphics (SGI) workstations and a Unix-based SGI Power Challenge 10 node parallel computer obtained through an earlier shared instrumentation grant from NSF which served 8 PI's in the Biochemistry and Biophysics Department. He currently administers an Unix-based SGI Altix 6-CPU cluster and a cluster of Linux-based 4 CPU/GPU workstations, to which he devotes about two calendar months effort (16% total effort) as part of his salary service duties to the Biochemistry and Biophysics Department. He thus has extensive experience administering Unix and Linux based computers. Dr. Sharp will continue to devote this level of effort to administering the requested equipment. Experience administering the previous two clusters demonstrates this is both feasible and adequate.

Dr. Sharp will have technical assistance and advice from the other two members of Operational Board, Drs. Williams and Axelsen (See Section E. Administration). The requested hardware has a Linux-based operating system, and all three are experienced Linux system administrators. Dr. Williams, who is director of the Cryo-EM core, currently administers the Linux workstation serving the data collection needs of that core. Dr. Axelsen previously administered a Linux based cluster of 8 Intel CPU's with Myrinet networking in the Pharmacology Department. Collectively the Operational Board either use, or are familiar with, all the major software packages to be run on the requested equipment. They will also be able to get technical advice, if necessary, from the primary major user of any particular software package. Given that all the software to be used is already currently installed and in use on at least one of the Operational Board's workstations or one of the Major User's workstations, we are confident that we have the technical expertise to set up and run the system and software end of the requested facility and to use it to make an immediate impact on SBMB research at the University of Pennsylvania.

B.3. Training

The requested resource is designed to be used intensively by a medium number of experienced major users, 13 in number, who are already running the same types of computations on a patchwork of existing workstations. There is therefore no anticipated overhead in training 'new users' or performing 'service' computations for Major Users. For Minor Users, their access is indirect, via their use of the Cryo-EM core (the the electron microscope resource lab or EMRL) and the director of that facility, Dr. Williams. The EMRL facility will provide assistance to their users who are performing 3D reconstruction, and EMRL will maintain the software for their work.

B.4 Data Access

Given the anticipated size of the data sets to be processed by the requested resource, the most efficient way to put data on and take it off is through hot swappable external 4Tb or 8Tb disk drives, a procedure requiring no technical expertise. The requested hardware has this capability. These drives currently cost about $20 per terabyte and would represent a modest expense for the Major Users. Minor users producing data via the Cryo-EM core already receive their data this way, so again, the requested facility will involve no new equipment or procedures.

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Continue on to Research Projects

***Instructions for Research Projects (30 pages max)**:* In this section, describe the benefit of the requested instrument to enhance research projects. You can divide this section into subsections Research Projects of Major Users or Specific Research Topics. The latter format may be especially useful to avoid redundancies in the presentation of research projects if several Major Users pursue research topics which follow similar protocols and scientific benefits of the new instrument for their projects are comparable. All Major Users must have substantial need for the requested instrument. Detailed eligibility requirements for Major Users are described in Section III 3. In addition, if there are Minor Users and other users, include a subsection Minor Users' Projects.

- Since the projects have been previously peer reviewed, describe their details only as necessary to explain how the requested instrument will advance the projects' research objectives. (Do not simply copy the Specific Aims section from a funded application.) Present sufficient technical details about types of samples or specific experimental protocols to be employed to allow evaluation of whether the instrument is appropriate, would be effectively utilized, and would provide advantages over other methods and other similar existing or new instruments. In particular, explain the need for special features and accessories of the requested instrument by describing the specific studies that will utilize these options as at least three Major Users must need any of these special options. Preliminary data are not required, but if available, they may be used to illustrate the benefit of the requested instrument to the research projects. Describe how generated data will be handled and analyzed so that benefits of the entire experimental set-up can be judged. Summarize benefits that the requested instrument will provide towards answering specific scientific questions. Be succinct and clear.
- If you choose to divide this section into Research Projects of Major Users subsections, list the PD/PI's name and grant information (number, title, project start and end dates) in the beginning of each subsection.
- If you choose to group research projects in subsections Specific Research Topics, in the beginning of each subsection list Major Users, their funded grants that you describe therein, and their cumulative usage as measured by the percentage of the AUT.
- Conclude this Research Projects section with a subsection Minor Users' Projects to describe the need of the requested instrument to advance projects from Minor Users and the user community at your institution (e.g., unfunded users who have significant need for the instrument to develop their research programs or users whose expected needs are at the level of 1% or less of AUT).
- In cases of certain technologies (such as computer systems or X-ray detectors), a large number of users, exceeding what is necessary to make a strong case for the need of the instrument, may be expected. In such cases, you may select a representative smaller group of Major Users and describe their research projects' needs in detail in subsections Research Projects of Major Users. Then, devote a separate subsection Other Users' Projects to describe research and instrumentation needs of your large user community, including Minor Users'. Keep in mind that the sole number of users is not a compelling factor to justify scientific needs for the requested instrument.
- You must focus this Research Projects section on detailed explanation of how the requested instrument will advance research projects. Research projects may be drawn from a broad array of topics in basic science, translational investigation or clinical trials; in particular, research projects on advancements of technologies for the benefit of biomedical research may be included. Demonstrate that NIH-funded investigators will use the instrument at the level of at least 75% of AUT.

- **Section Guidelines:** As adapted from the ACSB, this section should include the following: This section should begin with a brief summary of the major-user group—the schools, departments, and universities involved—and should state the broad use and support the instrument within the research community. List the major users first (project descriptions of two to three pages), then minor users (abbreviated project descriptions – one paragraph each is enough). Each research project should be organized as follows

    1. PI name and title, PI role, and project title
    2. One to three specific aims
    3. Background and significance
    4. Preliminary results that validate the need, use, and application of the requested equipment.
    5. Experimental procedures and protocols to demonstrate your understanding of the use of the instrument and potential difficulties.
    6. Use, application, and need for the requested instrument (including any accessories and unique capabilities) in fulfilling specific aims.

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Goldman –Research Projects

Major User Research Project

C.1.b. Axelsen, Paul H., Professor of Pharmacology

Grant Numbers and Titles:

R01-GM076201, Structure Determination by Vibrational Spectroscopy
R01-NS074178, Oxidative Lipid Stress in the Brain

Alzheimer's disease is characterized by an as-yet-undefined process occurring in the vicinity of amyloid plaques that causes neuronal dysfunction and death. These plaques consist largely of amyloid β (Aββββ) peptides that have aggregated into fibrils. Despite intense study, the structure of these fibrils and the factors that induce their formation are unknown. However, it clear that the fibrils we prepare in vitro vary widely in molecular structure, as well as in their thermodynamic stability, and that fibril structure can "mature" over time.1

Project: The dye-binding mode of amyloid fibrils and their basis for specificity

We are currently engaged in a multi-faceted study of the way in which supposedly amyloid-specific fluorescent dyes bind to amyloid fibrils – with less-than-ideal instrumentation. There is more belief than data on this topic, and more long extrapolations from model systems than solid data obtained directly from amyloid fibrils. The role of MFD-FRET in this project is to determine the orientation of bound (immobilized) acceptor fluorescent dye molecules with respect to freely rotating donor fluorophores on the fibril axis. The orientation of the plane of the acceptor (parallel to the fibril axis, or perpendicular) will have a profound effect on its rotational rate, which should be straightforward to measure with MFD-FRET. The question of

orientation is important because reliable and detailed molecular structures are not available for fibrils, yet amyloid-specific binding is the basis of diagnostic imaging tests for Alzheimer's disease. The repeating nature of amyloid fibril structure means that knowing whether dyes bind perpendicular or longitudinal offers important insight into what elements on the fibril amyloid-specific dyes are so specifically recognizing. With present instrumentation, we are limited by sensitivity and contrast against background because of sample requirements.

Specific Benefits of the Proposal/Equipment: Multiparameter fluorescence detection single-molecule FRET instrumentation would be an invaluable enhancement of our capability in these investigations because the range over which MFD-FRET measurements are informative (10-40 Å) is ideal for answering these questions, and far better than currently available instrumentation in which our samples are immobilized and subject to overwhelming amounts of background fluorescence. The current project will be much more elegantly performed by exciting bound dye molecules with donor fluorophores attached to the fibril, since that would dramatically reduce signals from nonspecifically excited background fluorescence. We routinely prepare fibril "seeds" that embody all of the structural features of full-length amyloid fibrils. With dimensions that are roughly 10 nm in diameter and 100 nm long, they readily diffuse in solution,2-8 and bound fluorophores will exhibit markedly different rotational rates depending on their orientation relative to the long axis, detectable using the polarization and anisotropy capability of the instrument.

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Minor User Research Project

C.2.a. Deutsch, Carol, Professor of Physiology
Grant Number and Title:
R01-GM052302 Biogenesis of Voltage-Gated K+ Channels

Project: Peptide Folding in the Ribosome Exit Tunnel

Protein synthesis involves a 2-way dynamism between the nascent peptide being elongated in the ribosome and the ribosome's exit tunnel (Fig. 16). This specialized microenvironment is a tight squeeze for a nascent peptide, and it likely contains sensors and signaling mechanisms for peptide folding. We have found diverse functional zones along the tunnel1, 2, 3 and that relocation and/or reorientation of the nascent peptide (both short-range and long-range) relative to the tunnel depends on the nature of the primary sequence of the nascent peptide3. We suggest that these discoveries reflect a multiplicity of peptide conformations and trajectories, which underlie signaling between different tunnel regions during translation. The multi-parameter fluorescence detection of FRET signals will allow identification and analysis of individual species present in our pool of nascent peptides attached to the ribosome, with a time-resolution amenable to translation events and transit through the tunnel.

Using MFD-FRET, we will initially i). test the hypothesis that different nascent peptides move along different tunnel pathways, ii). test the hypothesis that secondary structure of a nascent peptide in the tunnel can be recon-figured by its emergent N-terminus, and iii). determine the nature of putative 'compact' (helical?) structures in different regions of the tunnel.

To this end, the optimal fluorophores for intramolecular MFD-FRET can be covalently coupled to our nascent peptides either via our arsenal of strategically engineered cysteines1, 3, 4 or through our synthesis and in-corporation of unnatural amino acids (Po and Deutsch, unpub. data). Fluorescently-tagged residues are ac-commodated in the ribosomal tunnel for ensemble FRET measurements5 and probe-tagged cysteines easily transit the tunnel during translation and fold correctly1, 3, 6.

Specific Benefits of the Proposal/Equipment: The proposed single molecule FRET instrument will be important to advance these studies because we expect significant heterogeneity among partly translated peptides and a given peptide sequence may have a distribution of pathways. Ensemble FRET measurements cannot resolve these possible natural variations in the population. Distance measurements that delineate the peptide secondary structures within the exit tunnel and thereby folding during translation need to be accurate to make clear interpretations. The capability of the MFD-FRET instrument requested to quantify probe mobility, relative probe orientation, donor lifetime and quantum yield during individual measurements, distributions of these variables, and associated analysis software are essential for obtaining quantitatively reliable, calibrated distances be-tween the labeled residues. The MFD-FRET measurements will thus complement and extend our unique and ongoing studies of co-translational folding.

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Research Projects – Gupta Example

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).

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Research Projects – Litt Example

Major

Research Project

PI Name: LITT, BRIAN

Grant:
R01NS0993480 9/01/16 – 05/31/21

NIH/NINDS (P.I. Litt)
Title: Virtual Resection to Treat Epilepsy
Summary: In this proposal we develop a new method to localize epileptic networks from intracranial EEGthat: (1) replaces manual marking with an automated, objective method, (2) removes the need for precipitating acute seizures during evaluation to localize them and (3) allows clinicians to simulate the effects of different brain surgeries or device placements for individual patients in order to select the treatment that will work best for them.
Anticipated Usage*:* 2,500 hours annually (note, the Litt Lab will collect all available iEEG data)

Description:

The main goal of this research is to develop new, less invasive and more effective methods for guiding surgery, laser ablation and implantable device interventions to treat patients with medication resistant epilepsy. A very promising method we have developed is to build computational models of epileptic networksto guide these interventions, based upon intracranial recordings co-registered to high resolution brain imaging in patients undergoing grid, strip and stereo EEG invasive monitoring during evaluation for epilepsy surgery. To date our group has developed tools that can predict: (1) whether a patient is likely to benefit from focal resection or laser ablation, and (2) to quantitatively identify network hubs whose stimulation or resection is likely to be associated with seizure freedom, and (3) to track the spread of seizures in epileptic networks using neurostimulation co-registered to high resolution imaging of brain structure. This work is in collaboration with Dr. Danielle Bassett, a MacArthur Award winning computational neuroscientist, who is Co-PI on our parent grant, Kathryn Davis and Timothy Lucas, head of Penn's Epilepsy Surgery Program. Together, Drs.Bassett, Davis, Lucas and I are also developing methods to identify targets for implantable device deployment to improve patient outcome. In our research we will use the high channel count of the Blackrock system to record from these new high-resolution implanted electrode devices, and also to apply electrical stimulation, closed loop, to increase the resolution of network mapping far beyond the capabilities of our clinical system. It is anticipated that we will use this system, alongside our clinical system, which has a much lower channel count and is not capable of closed loop, controlled stimulation. We plan to record almost continuously from the Blackrock system for each implanted patient, when the system is not in use for specific research protocols deployed by other groups, and to use other groups' resting data as well, for our studies. Our estimate is that we will record a minimum of 35 patients per year, each continuously for a minimum of 7 days, with breaks for other studies, for a utilization of > 5,000 hours/ year. Having the Blackrock Neuroport System will fundamentally improve our ability to map patients for epilepsy surgery and to develop new strategies to target implantable devices at high resolution. It will also help us and to better understand the fundamental topology of epileptic networks and seizure generation. We plan to aggressively translate these findings into clinical care in the renewal of our parent R01, which is to be resubmitted in early July 2020. The Litt Lab has had multiple publications relevant to the BlackRock system as referenced below. Figure 1 from a recent paper by our group in BRAIN (Kini et al., Oct 2019) this year demonstrates findings from applying our Virtual Cortical Resection model to a patient with neocortical epilepsy and how this might change clinical care. The method suggests that broad resection of seizure generating regions may actually contribute to worse outcome when "desynchronizing" regions that oppose seizure propagation are removed in addition to synchronizing areas. Our plan, using the requested equipment, is to map these regions at much higher resolution, using high resolution intracranial grids, stereo IEEG and novel hybrid electrodes, that will better guide our models and eventually clinical translation of our results into clinical care.

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Research Project

PI Name: KAHANA, MICHAEL J.

Grants: R01 MH055687 (04/01/1997 – 01/31/2021) (PI Kahana) R01 S106611 (06/01/2019 - 05/31/2024) (PI Kahana) U01 NS113198 (09/15/2019 - 06/30/2024) (PI Kahana)

Title: How single neurons in the human brain encode space and time

Summary: We will carry out fundamental experiments to elucidate the mesoscopic and microscopic neural dynamics underlying human memory and use direct brain stimulation as a manipulative tool to study those dynamics. Additionally, we seek to create a dynamical timeseries model that predicts the evolution of brain activity during cognitive tasks and incorporates the effects of stimulation-induced perturbations on the system.

Anticipated Usage*:* 100 hours annually

Description: Time is a central dimension along which autobiographical memories are organized. For example, events that occur near each other in time are likely to be subsequently remembered together (Kahana, 1996), and people can recall the dates of many events from their lives with remarkable accuracy, years and even decades after they occurred (Burt et al., 2001; Janssen et al., 2006). Although the role of time in memory has been subject to extensive behavioral investigation (Friedman, 1993) and computational modeling (Howard and Kahana, 2002; Howard et al., 2015), the neural basis of this relationship remain unknown. Noninvasive functional neuroimaging studies in healthy subjects and studies of patients with focal brain lesions indicate a critical role for the hippocampus and surrounding medial temporal lobe (MTL) regions in remembering the order of experienced events (Mayes et al., 2001; Palombo et al., 2019; Lehn et al., 2009; Montchal et al., 2019). Recently, neurons in the rodent hippocampus have been reported to encode information about time in a similar manner as hippocampal “place cells” encode spatial information, with single neurons firing at selective times within a learned event structure

(Pastalkova et al., 2008; Eichenbaum, 2014). These findings suggest that internal representations of time and space may converge in thehippocampus, where they are translated into a common neural code that supports memory for *where* and *when* events transpired.

Here, we propose to investigate how single neurons in humans encode information about time together with spatial location in a task that requires both temporal and spatial memory. We will record single-neuron activity and local field potentials directly from the hippocampus and surrounding MTL regions in epilepsy patients who are implanted with intracranial electrodes for clinical seizure monitoring. Subjects will play a first-person navigation game called *Goldmine*, in which they explore a virtual environment under timed conditions while searching for gold pieces whose spatial locations they must remember (**Fig. 1, Kahana**). This task was designed to mimic many aspects of time coding experiments in rodents to allow direct comparisons with these studies to be made. In addition, we will be able to decouple temporal from spatial information to analyzedistinct neural responses to each of these variables. We will explore the following hypotheses:

1. Do hippocampal neurons encode information about time, independently from space, as subjects navigatethrough a virtual environment under fixed, timed conditions? Similarly, how do neurons in extrahippocampal regions represent temporal information?
2. Do experimental manipulations aimed at biasing subjects to attend to temporal vs. spatial information alter the degree to which hippocampal neurons represent these types of information, and do these manipulations also alter the degree to which memories are recalled in a temporally- or spatially-clustered manner.

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Research Project

PI Name: YOSHOR, DANIEL

Grant:
R01 EY023336 09/01/13 – 1/31/24 NIH/NEI (PI Yoshor)

Title: Visual Form Perception Produced by Electrically Stimulating Visual Cortex
Summary: The Yoshor laboratory will record and stimulate from intracranial electrodes semi-chronically implanted in patients with medically intractable epilepsy to ask fundamental questions about how visual information is processed in human subjects. Electrical stimulation of miniature research electrodes located over visual cortex in these subjects will be used to develop and test cortical stimulation protocols for in visualcortical prosthetic (VCP) devices for blind subjects.
Anticipated Usage*:* 100 hours annually

Description:
The high channel capacity of the Blackrock recording system will allow us to obtain simultaneous recordingsfrom high density arrays of mini-electrodes located over early visual cortical areas (Figure 1A). Using these recordings, we will determine the receptive fields (Yoshor et al 2007; Figure 1B) and other response properties of individual sites in visual cortex, as well as the overall structure of the map of visual space in human visual cortex with unprecedented spatial resolution. Electrical stimulation of visual cortex is known to produce perception of a small flash of light known as a phosphene. Our laboratory has many years of experience with electrical stimulation of human visual cortex (Murphey et. al. 2008, 2009; Beauchamp et. al. 2012; Bosking et. al. 2017a, b; Beauchamp et. al. 2020), and we will continue to investigate parameters which lead to more effective and efficient stimulation of visual cortex to produce visual percepts. The Blackrock Cerestim system will give us great flexibility in delivery of electrical stimulation patterns to one or more of the implanted electrodes. Characterizing the importance of various

parameters of electrical stimulation for the generation of phosphenes when single electrodes are stimulated is a necessary step in the development of safe and effective visual cortical prosthetics. We will investigate the importance of waveform shape, frequency, and other parameters on both the threshold for generation of phosphenes, andthe properties of the phosphenes reported by the subjects. For example, we currently have limited qualitative reports from blind subjects who prefer the visual experience generated by higher frequency stimulation (120Hz compared to 20 Hz). The Cerestim package will allow us to quantitatively examine the importance of this and other parameters in sighted subjects. Although understanding the parameters which impact the generation of phosphenes for individual electrodes is important, our main focus is in understanding how to generate perception of coherent visual forms using patterned stimulation of multiple electrodes in visual cortex. In previous work, we have found that concurrent stimulation of a set of surface electrodes in visual cortex is not sufficient to produce the perception of a coherent visual form. Instead, with this type of stimulation the subjects tend to see only a number of discrete individual phosphenes or patches of light (Bosking et. al. 2018). In both sighted EMU subjects (using the Blackrock Cerestim system), and blind subjects (implanted with a prototype VCP), we have found that if we instead use a rapid dynamic sequence of electrical stimulation of the same set of electrodes, that we can effectively communicate specific shapes or letters to the subject (Beauchamp et. al. 2020; Figure 1C; Figure 2). We will now systematically investigate how various parameters of the dynamic stimulation sequence impact the coherence or clarity of the forms that the subjects perceive. A high channel recording and electrical stimulation system is crucial to these experiments. The Blackrock system allows us to pick up to 96 electrodes that can easily be addressed and included in different dynamic stimulation sequences.

As an extension of our investigations of how to use electrical stimulation to produce useful visual form perception, we will directly compare integration of spatial patterns that are delivered either through natural visual stimulation of the retina, or through direct electrical stimulation of visual cortex. During these experiments the Blackrock system will allow us to record simultaneously from multiple visual areas to assess how the two input methods produce different propagation of signalsthrough cortical networks. Another focus ofour laboratory will be to assess the role of alpha rhythms in visual cortex. In particular, it has been proposed that suppression of these ongoing rhythms is essential for visual detection, and that presentation of visual information might be most effective when presented at particular phases of the ongoing oscillations. The Blackrock system will permit us to use high density recordings over primary visual cortex obtained while subjects perform visual detection tasks to better assess the impact of these ongoing rhythms on subject performance. We will use closed-loop electrical stimulation delivered through the Blackrock Cerestim system to disrupt or modify ongoing cortical activity to test for a causal role of alpha rhythms in visual perception and spatial attention.

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Research Project

PI Name: BASSETT, DANIELLE, S.

Grant:
NSF IIS-1926757
09/01/19 – 08/31/23
NIH/NIDCD (P.I. Bassett)

Title: Validity and Utility of Mathematical Models of Brain Network Function and Control
Summary: The main aim of this project is to test prediction from network control theoretic models about how neural activity changes in response to electrical stimulation and neurofeedback.
Anticipated Usage: 60 hours annually

Description
Network control theory is an emerging field of systems engineering that uses formalisms from mathematics, physics, and engineering to expand upon descriptive observations of different brain states and address how subjects' mental effort or exogenous stimulation can drive the brain between those states. This framework shows potential for insights into a variety of cognitive domains by providing models and hypotheses for modulating brain activity via neurofeedback or stimulation. Most of the hypotheses have not been tested yet, and would require the Blackrock system to design and implement custom simulation paradigms with high quality electrocorticography recordings. Previous work in the lab used a combination of biophysical models (Kuramoto oscillators) and empirical electrocorticography to model how direct electrical simulation spreads across white matter connections in the brain to affect distant region's activity. Metrics from this model associated with white matter connections identify which regions are theoretically better suited to steer the brain to different activity patterns. Validation with publically available stimulation data show that these metrics predict the effects of direct electrical simulation in humans. Further validation of predictions from these models, and further exploration of how the system of white matter connections influences activity spread will require us to design our own simulation paradigms that use multiple simulation sites for each patient. The availability of a Blackrock "CereStim" microstimulator system at Penn will be critical for implementing these experiments. Other work in our lab on endogenous control has shown that the development of white matter networks over adolescence facilitates efficient control of the fronto parietal network. The next step in this research would involve testing whether the same model also explains the efficacy of neurofeedback to regions of

the frontoparietal network. This experiment requires that subjects' brain activity be immediately processed and displayed to the subject, allowing them to modulate their own brain activity in a closed loop (Fig. 1, Bassett). The real-time, state-of-the-art Blackrock NeuroPort system is required for this presentation. These studies could serve as the foundation for future applications to facilitate learning, cognitive control, and other high-order functions that rely on interacting cortical circuits. The high-quality data acquisition and real-time monitoring capabilities of this device are crucial for this research.

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Research Project

MPI Names: PROEKT, ALEXANDER & LUCAS, TIMOTHY H.

Grant:
R01NS113366
06/01/20 – 05/31/25
NIH/NINDS
Title: The role of dynamical criticality in human perception
Summary: The proposed research will establish a new and theoretically-sound property of neuronal activity that allows the brain to consciously detect and respond to sensory stimuli. Identifying the conditions in whichthis property is present will help us reliably determine the likelihood of conscious perception in patients undergoing anesthesia and in those suffering from brain injury.
Anticipated Usage*:* 120 hours annually

Description:
The human brain possesses a remarkable, but notably fluctuating, ability to detect and respond to stimuli. Sensory responsiveness is greatly diminished during drowsiness and sleep. Under general anesthesia, even noxious stimuli are not perceived. The mechanisms by which perception is modulated in the anesthetized, sleeping, and waking brain remain poorly understood. In this project, we will test a novel theory linking perception across arousal states to a common feature of neuronal dynamics. Specifically, we hypothesize that sensory responsiveness is attained only if the neuronal dynamics are poised at a bifurcation between stable and unstable oscillations. We have previously found evidence for this criticality hypothesis using time- resolved multivariate autoregression modeling of neural signals recorded in nonhuman primates and in a pilot study with human subjects. In this project, we will comprehensively test this hypothesis by quantifying the stability of neuronal dynamics, estimated from electrocorticography (ECoG) signals in human subjects undergoing epilepsy surgery, during pharmacologically-altered sensory responsiveness, as well as during naturally-occurring diurnal, and ultradian fluctuations in sensory responsiveness (Fig. 1, Proekt & Lucas).

There are two aspects of the project that would benefit tremendously from acquisition of the proposed Blackrock system. First, our prior work has indicated that robust estimation of network dynamic stability using multivariate autoregression modeling is dependent on a large number of simultaneously recorded neural signals with low noise. The higher channel count (512 channels), superior dynamic range, and lower noise floor of the Blackrock NeuroPort system compared to our current clinical (Natus) system should improve our ability to detect changes in dynamical stability. Second, a key prediction of the criticality hypothesis is that stabilization of ECoG dynamics leads to spatiotemporal dampening of stimulus-evoked responses. Thus, an important test of the hypothesis will be to deliver electrical stimuli and quantify how the evoked responses vary in relation to the estimated stability metric. At present, however, Penn does not have the capability of delivering electrical stimuli in the operating room where we will be pharmacologically manipulating brain state. The Blackrock CereStim would provide this capability and

thus dramatically improve our ability to test the hypothesis.

Fig 1, Proekt & Lucas: Neuronal dynamics stabilize when consciousness is suppressed with mechanistically distinct anesthetics. A–C, Ketamine–medetomidine; **D–F**, propofol. Evolution of the distribution of the criticality indices as a function of time since anesthetic injection for monkey M2 under ketamine–medetomidine (**A**, top) and under propofol (**D**, top). For each time window, the probability distribution of the criticality indices is shown by color (red shows low probability, yellow shows high probability). Note that, in the awake state, criticality indices crowd the critical line (∼1) between stable (<1) and unstable (>1) regions. With drug injection, the fraction of criticality indices above 0.98 drops abruptly (**A**, **D**, bottom). Results obtained for monkey M2 are representative of recordings in all monkeys (see Figs. 3, 4). **C** and **F** show the average drop in the number of the most critical modes 4 min after ketamine–medetomidine and propofol, respectively. The differences in the distribution of criticality indices after drug injection are statistically significant in all monkeys (**B**, **E**): p value of a Kolmogorov– Smirnov test comparing criticality indices at t = 0 and at subsequent times. **p < 0.01.

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Research Project

PI Name:

GOTTFRIED, JAY A.

Grant:
R01DC018075
04/01/20 – 03/31/25
NIH/NIDCD (P.I. Gottfried)
Title: Spatiotemporal Mechanisms of Olfactory Processing in the Human Brain.
Summary: The major aim of this research project is to use intracranial recording techniques in human patients with medically intractable epilepsy to elucidate the spatiotemporal mechanisms of odor processing in the human olfactory system.
Anticipated Usage*:* 60 hours annually

Description:
A primary focus in our lab is to characterize the electrophysiological determinants of odor perception directly from the human brain, in epilepsy patients with medically resistant seizures. These rare patients offer a unique opportunity to record intracranial EEG (iEEG) activity from olfactory-related area in the medial temporal and basal frontal lobes, including piriform cortex, amygdala, hippocampus, and orbitofrontal cortex (OFC). In initial work, we found that natural breathing (in the absence of sniffing or smelling) sets the pace of slow respiratory oscillations in human PC, and also entrains higher-frequency oscillations in PC, amygdala, and hippocampus. These effects strikingly dissipated when breathing was re-directed from the nose to the mouth. A recent publication demonstrates that in the presence of odor, oscillations of piriform local field potential (LFP) activity emerge in the theta-frequency band, and can be used to decode stimulus-specific olfactory content as quickly as ~110 ms post-sniff. We have also made considerable progress on a new iEEG study examining olfactory working memory across sets of three sequential odors, with preliminary results suggesting that odor-induced theta is followed by gamma oscillations that persists throughout the maintenance phase between sniffs. Going forward, we are very interested in developing two complementary research goals to enhance our neurophysiological understanding of the human olfactory system. Our first goal is to conduct paired (hybrid) macro-electrode and micro-electrode recordings, such that we can make neuron-level inferences from population-based LFP activity. Thus, availability of a real-time, state-of-the-art Blackrock NeuroPort system would be ideal for collecting single-unit data with low noise and high fidelity. Such equipment is currently not available at Penn. Our second goal is to conduct microstimulation experiments on the human olfactory system, targeting the piriform (olfactory) cortex, amygdala, and hippocampus, all of which receive dense input from the olfactory periphery. This approach will enable us to establish whether focal stimulation trains

can disrupt odor perception, bringing causal understanding to the role of these brain areas on olfactory network coding. Thus, availability of a Blackrock CereStim microstimulator system would fundamentally transform our ability to gain both basic and clinically focused insights about the relevance of such brain regions to odor perception and discrimination.

Our framework for inferring basic principles of odor coding from theta activity in human piriform cortex (PC) centers on the idea that the LFP signal arises from temporally organized activity across local ensemblesof neurons. Thus, by measuring odor-induced perturbations in theta features, we can begin to delimit the mechanisms by which synaptic currents of piriform cortical ensembles give rise to changes in the LFP. For example, changes in theta amplitude could arise from stronger responses in local PC neurons and/or from increased temporal synchrony across neurons (Fig. 1, Gottfried). However, without direct recordings from individual neurons, fine-scale mechanistic insights are not possible. Availability of the Blackrock system will enable us to obtain simultaneous recordings of single neurons and LFP's, helping establish the causal electrophysiological relationships that underlie odor processing in human olfactory cortex.

A singular advantage of iEEG techniques is the ability to deliver electrical stimulation directly to specific brain regions while patients perform different cognitive tasks. Prior iEEG studies have extensively examined language, motor, and memory functions in the human brain, but few studies have been conducted in human PC, without any attempt to demonstrate the causal involvement of PC (or PC theta oscillations) in odor processing. We have recently designed a carefully controlled electrical stimulation study involving reversible perturbation of the PC, to establish the causal role of PC theta oscillations in odor perception. Preliminary data (Fig. 2, Gottfried) shows that PC theta stimulation induces robust theta oscillations in lateral PC, and the identification of consistent phase lags between PC and PC-lateral PC suggests that these oscillations reflect a physiological effect of synaptic entrainment, rather than volume conduction per se. Using an advanced data acquisition system such as the Blackrock, representing the state-of-the-art system capable of delivering closed-loop stimulation upon detecting different neural states (e.g., rest state, ongoing theta, ongoing gamma) would allow us to elucidate the state-dependent role of theta oscillations in odor processing.

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Arabkheradmand, G., Zhou, G.,

Noto, T, Yang, Q., Schuele S.U., Parvizi, J., **Gottfried, J.A.**, Wu, S., Rosenow, J.M., Koubeissi, M., Lane, G, & Zelano, C. (2020). Anticipation-induced delta phase reset improves human olfactory perception. *PLoS Biology*, in press.

Research Project

PI Name: DAVIS, KATHRYN ADAMIAK

Grant:
K23 NS0929731
04/01/16 – 03/31/21 NIH/NINDS (P.I.
Davis)
Title: Localizing Epileptic Networks Using Novel 7T MRI Glutamate Imaging
Summary: This proposal applies a novel 7T MRI technique, GluCEST, that images the excitatory neurotransmitter glutamate at high resolution, to localize epileptic networks. Glutamate is widely believed to be central to seizure-generation, and preliminary data indicates that this technique can identify seizure generating regions in patients not-localized by other techniques.

Grant:
RF MH117188
09/01/18 -08/31/22
NIH (P.I. Halgren, Bazhenov, Cash; site-P.I. Davis)
Title: From ion channel dynamics to human EEG and MEG: multiscale neuronal models validated by human data (subcontract site)
Summary: The goal of this proposal is to build a neural model of EEG and MEG generation, linked via biophysical models to parallel empirical measurements, at multiple levels from trans-membrane currents to whole-brain non-invasive measures and a set of predictions regarding what specific features of the MEEG measurements tell us about underlying neuronal and synaptic dynamics.

Anticipated Usage*:* 2,500 hours annually (note, the Davis Lab will collect all available iEEG data)

Description:
The current “gold-standard” utilized to identify epileptic networks is intracranial EEG and the Davis lab is developing multimodal imaging methods and correlating the findings with intracranial EEG. The ultimate goal of this work is to identify epileptic networks noninvasively obviating the need for intracranial EEG. In initial work, we found that GluCEST can localize the hippocampus of seizure onset in temporal lobe epilepsy patients with nonlesional brain MRIs. In recent work, the Davis Lab has been directly linking the structural relationship as measured by diffusion imaging with intracranial EEG. Fig. 1, Davis illustrates the patient- specific structural connectivity-functional connectivity analysis pipeline. Thus, availability of a Blackrock "CereStim" microstimulator system would fundamentally transform our ability to correlate multimodal imaging with intracranial EEG. Our estimate is that we will record a minimum of 35 patients peryear, each continuously for a minimum of 7 days, with breaks for other studies.

Dr. Davis also is the site-PI for a NIH funded research project linking electroencephalogram (EEG) and magnetoencephalogram (MEG; together MEEG). MEEG are directly and instantaneously coupled to the currents across cortical neuronal membranes which mediate information processing. Numerous studies in vitro, animals and humans have largely identified the ligand- and voltage- gated currents, local and distant circuits underlying basic sleep and waking rhythms which dominate MEEG. Furthermore, methods exist to

estimate these rhythms in the cortex from MEEG. However, thisinformation is fragmented such that it is not possible to infer basic cortical neurobiological properties from MEEG. In this grant, we produce a detailed multi-scale model of how human MEEG are generated, including channels, synapses, neurons, structures, and networks as defined by the vast relevant scientific literature. In this project we compare the cortical cortical complex covariance matrix estimated from MEEG in a given subject measured pre-implant with the actual measured values from SEEG and use simultaneous EEG during SEEG to confirm the stability of the recordings. The availability of a Blackrock "CereStim" microstimulator system will substantially benefit the overall project. Our estimate is that we will record a minimum of 35 patients per year, each continuouslyfor a minimum of 7 days, with breaks for other studies.

Bibliography & References Cited: Select relevant references involving iEEG are noted below. Note that *Dr. Davis is a co-author on >30 other publications utilizing iEEG (the majority of which utilized the BlackRock System through the DARPA Restoring Active Memory project).* See complete list of published work on PubMed (link in Biosketch).

Conrad EC, Tomlinson SB, Wong JN, Oechsel KF, Shinohara RT, Litt B, **Davis KA***, Marsh ED*. Spatial distribution of interictal spikes fluctuates over time and localizes seizure onset. Brain. 2020 Feb 1;143(2):554-569. doi: 10.1093/brain/awz386. PubMed PMID: 31860064. (*= co-last-authors)

Kini LG, Bernabei JM, Mikhail F, Hadar P, Shah P, Khambhati AN, Oechsel K, Archer R, Boccanfuso J, Conrad E, Shinohara RT, Stein JM, Das S, Kheder A, Lucas TH, **Davis KA**, Bassett DS, Litt B. Virtual resection predicts surgical outcome for drug-resistant epilepsy. Brain. 2019 Dec 1;142(12):3892-3905. doi: 10.1093/brain/awz303. PubMed PMID: 31599323; PubMed Central PMCID: PMC6885672.

Shah P, Ashourvan A, Mikhail F, Pines A, Kini L, Oechsel K, Das SR, Stein JM, Shinohara RT, Bassett DS,Litt B, **Davis KA**. Characterizing the role of the structural connectome in seizure dynamics. Brain. 2019 Jul 1;142(7):1955-1972. doi: 10.1093/brain/awz125. PubMed PMID: 31099821; PubMed Central PMCID: PMC6598625.

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Ung H, Cazares C, Nanivadekar A, Kini L, Wagenaar J, Becker D, Krieger A, Lucas T, Litt B, **DavisKA.** Interictal epileptiform activity outside the seizure onset zone impacts cognition. Brain. 2017 Aug 1;140(8):2157-2168. doi: 10.1093/brain/awx143. PubMed PMID: 28666338; PubMed Central PMCID: PMC6167607.

Research Project

PI Name: COHEN, YALE E

Grant:
R01DC017690
06/01/19– 5/31/24
NIH/NIDCD (P.I. Cohen)

Title: Coincidence and continuity: uncovering the neural basis of auditory object perception.

Summary: The major aim of this research project is to understand the interactions (correlations) between mesoscopicpopulations of neurons and how these interactions contribute toauditory perception.
Anticipated Usage*:* 60 hours annually

Description:

Auditory objects are the foundational building blocks of our auditory-perceptual world. Auditory objects are formed, in part, by the brain's ability to extract and organize spectral and temporal regularities from the acoustic environment. In many cases, temporal regularities are formed across multiple frequency (Fig. 1, Cohen).

Bibliography & References Cited:

Gifford, A. M., Sperling, M. R., Sharan, A., Gorniak, R. J., Williams, R. B., Davis, K., Kahana, M. J., & Cohen, Y. E. (2019). *Neuronal phase consistency tracks dynamic changes in acoustic spectral regularity.* The European journal of neuroscience, 49(10), 1268–1287. https://doi.org/10.1111/ejn.14263

Minor

Research Project

PI Name: Gold, Josh I.

Grant: Internal Penn Funding & T32 trainee, Ashwin Ramayya (T32 NS091006)
Title: Neural mechanisms underlying adaptive decision making in the human brain
Summary: We aim to use intracranial EEG recordings from patients with medically refractory epilepsy to study the neural mechanisms that underlie adaptive decision making
Anticipated Usage: 60 hours annually

Description:
When faced with similar options, individuals take longer to make decisions (Naatanen and Niemi, 1981). This ability to deliberate, or delay choice in the face of conflict, allows one to more thoroughly evaluate options and make better decisions (Gold and Shadlen, 2007). Pathological behavior in addiction and problem gambling is thought to result from dysfunction in the ability to resolve conflict between immediate temptations and long-term goals (Mcclure and Bickel, 2014). In the proposed studies, we will obtain intracranial electroencephalograpy (iEEG) recordings from patients with drug refractory epilepsy as they make decisions with varying levels of conflict. Models of decision making and non-human primate electrophysiology studies

suggest that variability in reaction times can be explained by two major sources of variability: 1) the rate at which neural firing activity in distributed motor-related regions reaches threshold activation, 2) variability in the amount of change required for distributed motor-related activity to reach the threshold activation (i.e., variability in baseline activity at the time of stimulus presentation or threshold activation level at the time of response (Hanes and Schall, 1996; Gold and Shadlen, 2007). We will leverage the high spatial and temporal resolution of iEEG data along with its unique ability to simultaneously measure local and global neural activity to better the neural mechanisms underlying reaction time variability seen during deliberation (Ramayya et al., 2015). **In our first aim, we will study the relation between distributed motor-related activity and reaction time distributions during various degrees of conflict.** We will test the hypothesis that trial-by- trial variability in ramping of motor-related activity underlies stochastic variability in reaction time distributions, whereas changes in baseline activity underlies systemic variability seen between different levels of conflict. The Blackrock NeuroPort system will allow us to obtain simultaneous micro-electrode and macro-electrode recordings and study distributed motor-related activity using two distinct, high fidelity measures – population-level firing rates from microelectrode recordings and high-frequency activity (70-200 Hz) from macroelectrode recordings. In our second aim, we will test causal relations between **distributed motor-related activity and reaction times during various degrees of conflict.** We will use the Blackrock "CereStim" microstimulator system to apply precisely timed microstimulation near motor-related neurons to test causal relations with reaction time distributions. We will test the hypothesis that stimulation during periods of ramping activity and baseline activity will have distinct effects reaction time distributions, consistent with changes in stochastic and systemic variability, respectively. Thus, the Blackrock system will specifically enable us to perform our proposed studies of adaptive human decision making.

Bibliography & References Cited:

Gold JI, Shadlen MN (2007) The Neural Basis of Decision Making. Annu Rev Neurosci 30:535–574 Hanes D, Schall (1996) Neural control of voluntary movement initiation. Science (80-) 274:427--430.

Ramayya AG, Pedisich I, Kahana MJ (2015) Expectation modulates neural representations of valence throughout the human brain. Neuroimage.

Research Project

PI Name: KELZ, MAX B

Grant: Translational Neuroscience Initiative
2/1/17-6/30/20
University of Pennsylvania Seed Grant (PIs: Kelz)
Title: Multi-Sensory Integration: A Neurophysiologic Correlate of Conscious Perception
Anticipated Usage*:* 60 hours annually

Description:

Every waking moment the brain effortlessly synthesizes information arriving from different sensory modalities and integrates these sensory streams to form unified percepts. How the brain solves this binding problem isnot clear. The neuronal correlates of basic psychophysical quantities such as detection thresholds can be studied in animals. The binding problems, however, is best studied in humans because only humans can directly communicate their subjective perceptions. Yet, most readily available modalities for recording of brain activity in humans lack either requisite temporal (e.g. fMRI, PET) or spatial (e.g. EEG, MEG) resolution. Thus,in this proposal we will take advantage of the unique opportunity to record brain activity using electrodes implanted into the brains of patients undergoing surgical treatment of epilepsy. To address the neuronal correlates of multisensory integration we will deploy a combination of

psychophysics approaches and pharmacological interventions. The psychophysics approach consists of creating multisensory illusions by presenting visual and auditory stimuli in close temporal proximity. Pharmacological approach consists of administering drugs that either enhance (e.g. ketamine) or diminish (e.g. propofol) multisensory integration.

We have already developed behavioral paradigms, and optimized anesthetic drug administration in a pilot study with healthy human volunteers using surface EEG. We are currently preparing to deploy these paradigms on epilepsy patients implanted with depth electrodes in collaboration with the Proekt and Lucas labs. I am an attending anesthesiologist and systems neuroscientist with established collaborations with Tim Lucas (Neurosurgery), Alex Proekt (Anesthesiology), Brian Litt (Neurology), Kate Davis (Neurology), and Diego Contreras (Neuroscience). Consequently, our group is ideally positioned to leverage a wealth of intellectual capital in an ideal environment at the University of Pennsylvania with immense benefits that the Blackrock instrument would provide.

The psychophysics experiment consists of a double flash sensory illusion. We have optimized this behavioral paradigm in human volunteers fitted with high density surface EEG. The use of precisely timed auditory stimuli bracketing a single visual flash can produce a subjective perception of two flashes. The subject reports whether they perceived one or two flashes in a standard two alternative forced choice design. This illusion depends critically on the integration of two sensory streams (auditory and visual) into a single percept. Its presence thus indicates the explicit occurrence of multisensory integration events. Because the illusion occurs only on a subset of trials (~ 50%), we can directly compare the evoked responses in trials when the illusion is present or absent to identify the electrophysiological features of the evoked response that are specifically associated with conscious perception while keeping the external stimuli identical. A slight modification of the paradigm (presentation of two flashes) allows us to directly compare illusory vs. non- illusory perception of two flashes. This control allows us to further refine the conjunction between brain activity and subjective perception. Using high density surface EEG recordings synchronized with the stimulus presentation and behavioral responses that indicate whether a subject saw one or two flashes, we have produced pilot data demonstrating the successful identification of spatiotemporal signatures of cortical brainactivity that are specifically related to conscious perception.

In existing Penn IRB-approved protocols, we have also developed target-controlled infusions in humans to achieve steady-state brain concentrations of anesthetic drugs with distinct mechanisms of action. We systematically vary the amount of propofol, a general anesthetic capable of eliciting dose-dependent sedationup through levels that produce an unconscious state, or of ketamine, a distinct anesthetic with a different mechanism of action that is capable of eliciting synesthesia at low doses and unconsciousness at high doses,to directly modulate arousal. As part of this S10 consortium grant, we propose to conducting parallel studiesin patients with intractable epilepsy who present for invasive EEG recordings. The ability to test multisensory integration in humans undergoing precise titration of general anesthetics to regulate arousal will yield unparalleled temporal and spatial resolution of the electrophysiological determinants supporting conscious perception.

Bibliography & References Cited:

Kelz MB, Mashour GA. (2019) The Biology of General Anesthesia from Paramecium to Primate.Curr Biol. 2019 29(22):R1199-R1210 PMC6902878

Morgan PG, **Kelz MB**. (2019) Be Wary of Genes Governing Awareness. Anesthesiology. 131(5):955-956.PMID: 31335547

Shortal BP, Hickman LB, Mak-McCully RA, Wang W, Brennan C, Ung H, Litt B, Tarnal V, Janke E, Picton P, Blain-Moraes S, Maybrier HR, Muench MR, Lin N, Avidan MS, Mashour GA, McKinstry-Wu AR, **Kelz MB**, Palanca BJ, Proekt A; ReCCognition Study Group. (2019) Duration of EEG suppression does not predict recovery time or degree of cognitive impairment after general anaesthesia in human volunteers. Br J Anaesth.123(2):206-218. PMC6676227

Hemmings HC Jr, Riegelhaupt PM, **Kelz MB**, Solt K, Eckenhoff RG, Orser BA, Goldstein PA. (2019) Trends Pharmacol Sci. Towards a Comprehensive Understanding of Anesthetic Mechanisms of Action: A Decade of Discovery.40(7):464-481. PMC6830308

Aggarwal A, Brennan C, Shortal B, Contreras D, **Kelz MB**, Proekt A.(2019) Coherence of Visual-Evoked Gamma Oscillations Is Disrupted by Propofol but Preserved Under Equipotent Doses of Isoflurane. Front Syst Neurosci. 13:19. PMC6519322

Li D, Vlisides PE, **Kelz MB**, Avidan MS, Mashour GA; ReCCognition Study Group. (2019) Dynamic Cortical Connectivity during General Anesthesia in Healthy Volunteers. Anesthesiology. 130(6):870-884. PMID: 30946055

Kelz MB, García PS, Mashour GA, Solt K. (2019) Escape From Oblivion: Neural Mechanisms of Emergence From General Anesthesia. Anesth Analg. 128(4):726-736. PMC6885009

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Sharp – Research Projects

Major User Research Project

PROJECT 1: STRUCTURAL BIOLOGY AND MOLECULAR BIOPHYSICS OF PROTEIN POST- AND CO-TRANSLATIONAL MODIFICATION AND CHROMATIN REGULATION

Investigator:
Ronen Marmorstein, Ph.D., Perelman School of Medicine, University of Pennsylvania
Professor, Department of Biochemistry and Biophysics
Investigator, Abramson Family Cancer Research Institute

Relevant NIH Grants:
R35 GM118090
R21 AI1126317

P01 AG031862 (P1)
P01 CA114046 (P3)

The Marmorstein laboratory studies the molecular mechanisms of protein post- and co-translational modification with a particular focus on protein acetylation and phosphorylation and chromatin regulation. The laboratory uses a broad range of molecular, biochemical and biophysical research tools centered on macromolecular structure determination using both X-ray crystallography and cyro-electron microscopy (cryo-EM). The laboratory is particularly interested in gene regulatory proteins and their upstream signaling kinases that are aberrantly regulated in cancer and other age-related disorders. They use high-throughput small molecule screening and structure-based design strategies to develop protein-specific small-molecule probes to be used for further interrogation of protein function and for development into therapeutic agents.

The projects in the laboratory that require significant computational resources are (1) structures of large macromolecular assemblies using single particle cryo-EM and (2) in silico screening of novel kinase and acetyltransferase inhibitors. (Figure 1)

In the area of large protein assemblies, the laboratory is studying how N-terminal acetyltransferases (NATs) carry out co-translational protein acetylation and how multi-protein histone chaperone complexes coordinate histone deposition into chromatin. N-terminal protein acetylation serves as one of the most common post-translational protein modifications, modulating many protein activities including cellular apoptosis, enzyme regulation, protein localization, rDNA transcriptional regulation and the N-end rule for protein degradation (1-4). Most eukaryotes contain 6 NATs (NatA through NatF) that differ in their substrate specificities and subunit composition. Over the last several years, the laboratory has used X-ray crystallography together with biochemical and enzymatic assays to characterize the structure-function relationship of NatA (5), NatD (6) and NatE (7), using fission yeast as a model system. Despite the information that has been obtained on these isolated NATs, how they associate with the ribosome to coordinate co-translational protein acetylation is not understood. Towards understanding this, the laboratory has been assembling NAT/ribosome complexes for single particle cryo-EM reconstruction. The laboratory prepared fission yeast ribosome and determined a preliminary cryo-EM reconstruction at ~4 Å resolution (Figure 1A). The laboratory is now preparing NAT/ribosome complexes for cryo-EM reconstruction, starting with the NatA complex (Figure 1B), but the laboratory will subsequently prepare complexes with other NATs.

The HIRA histone chaperone complex, composed of HIRA, Ubinuclein-1 (UBN1), and CABIN1, cooperates with the histone chaperone ASF1a to mediate H3.3-specific binding and chromatin deposition to mediate gene regulation and DNA repair (8-11). Over the last decade, the laboratory has dissected the molecular interactions within the complex, which are important for the histone H3.3 deposition process (Figures 2A and 2B). This includes X-ray crystal structures of Asf1/HIRA 12 and UBN1/H3-H4 (13) complexes and a biochemical characterization of HIRA/UBN1 (14) and HIRA/CABIN1 (15) interactions. The laboratory has begun to assemble the entire 4-subunit HIRA complex (Figure 2C) for cryo-EM reconstruction and has obtained some encouraging preliminary negative stain images (Figure 2D) towards a structure determination, which we will pursue over the coming years.

Over the last decade, the laboratory has carried out small molecule high-throughput screening (HTS) campaign both in vitro (16-19) and in silico (20-23) and structure-based inhibitor development employing structural, biochemical and cell-based studies (24-31). This has lead to inhibitors to the kinases BRAF, PI3K, PAK1, S6K1; the acetyltransferases NatA, NatE and p300; and the human papillomavirus oncoproteins E6 and E7. The laboratory is currently carrying out additional in silico screens against the NatA and hMOF acetyltransferases.

(Figure 2)

Together, the cryo-EM and in silico small molecule screening campaigns described above has relied on use of the Penn High Performance Computing (HPC) resources at the Penn Medicine Academic Computing Services (PMACS). **However, the jobs have been relatively low throughput on the typical number of cores available at any time at PMACS, and do not scale well with number of cores on the hardware that is available, requiring jobs to typically take on the order of days rather than what would be hours using the request instrumentation. In addition, access to the PMACS facility has been limited and wait times or jobs has been long (several days to weeks), which has significantly slowed scientific progress in the Marmorstein laboratory.**

Minor User Research Project

PROJECT 13. OTHER USERS' PROJECTS: Single particle reconstruction for researchers at the electron microscope resource lab (EMRL), Dewight Williams, Ph.D, core director.

Relevant NIH Grants of minor users

Relevant NIH Grants of minor users

Researcher	Institution	Grant number
Jeff Benovic	Thomas Jefferson Medical Center	RO1 GM044944
Gino Cingolani	Thomas Jefferson Medical Center	RO1 GM100888
Patrick Loll	Drexel University	P01 GM055876
Carol Deutsch	Department Physiology U. Penn.	RO1 GM052302
James Peterson	Department of Chemistry U. Penn	RO1 NS081033
David Christianson	Department of Chemistry U. Penn.	RO1 GM056838
Ivan Dmochowski	Department of Chemistry U. Penn.	RO1 GM083030
Emmanuel Skodalakes	Wistar Institute	RO1 GM088332

The Electron Microscopy Resource Laboratory (EMRL) at the University of Pennsylvania's Perelman School of Medicine is dedicated to providing EM services to researchers within the University and surrounding institutions. It is established as a recharge core facility but operated as a resource providing equipment and training to researchers so that they can access and conduct EM based research. This facility's ability to recharge for use distributes the high costs of EM instrumentation over many users RO1 grants within our home institution, as well as to neighboring institutions, and provides a mechanism to maintain and budget for new technology/equipment. The facility houses four electron microscopes (pathbio.med.upenn.edu/pbr/portal/) with a direct electron detector on a 200 KeV FEI Tecnai-F20. This instrument is our primary instrument for data collection of cryogenic preserved biological material and supports automated image acquisition of both tomographic and single particle datasets. The Falcon II direct electron detector outputs 18 frames a second image stacks to an ancillary computer system and these movie files are aligned during image acquisition using a Titan X™ Nvidia graphics processor and the motion correction software DOSEFGPU from the laboratory of Yifeng Cheng at UCSF (1). Currently, we have many NIH funded users not included in this proposal that are now collecting cryogenic TEM images of their favorite protein complexes with the long term goal of solving these structures by single particle reconstruction methods (See table 1). This work is generating 2 to 6 TB of data per day of use and we are currently experiencing a bottle neck in image processing capabilities. Further, it is anticipated in the coming year, the School of Medicine will purchase an FEI Titan Krios equipped with a Gatan K2 direct electron detector both to help recruit faculty and to achieve atomic resolution from our cryogenic TEM, SPR efforts.

The CMOS based direct electron detector's high read out rate has allowed motion correction of cryogenic transmission electron microscopic images containing biological assemblies which has led to atomic resolution structures for a large number of protein complexes since 2013 (2-7). The ability to reconstruct these 2D projection images into 3D structures at atomic resolution requires registration of individual particles in 10's of movie frames across a data set of images containing 10,000s to many 100,000s individual macromolecular complex images (8, 9). These images are then aligned to starting model of the structure or a de novo structure determined with common lines methods or Simple 2.0 software (10). This process comes with a high computational cost and requires a parallel high performance computational environment with high throughput disk read/write bandwidth to disk storage arrays that hold many hundreds of terabytes of data. Moreover, software such as Relion 2.0, GeFrealign v9.xx, NAMD molecular dynamics fitting, and DOSEFGPU motion correction are taking advantage of the computational power of graphics processing units that can increase vector based computation speeds many fold. **Establishing a cluster primarily designed and dedicated to these applications is essential for the timely structure determination of the users of the EM resource laboratory at the Perelman School of Medicine. Currently, data collection is delayed for many researchers due to the inability to free up space on the facility's disk arrays because the time to process single particle reconstructions. This has led to a backlog of possible structures. This proposal is to acquire a cluster that both contains a set of CPUs with enough memory to process large 3D volumes (500 Gb to 1 Tb of RAM) and is balanced with nodes that have K80 Nvidia Tesla™ graphics processors that will allow greater speed in molecular dynamics and 3D backprojection calculations (11-13).**

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Continue on to Summary Table(s)

***Instructions for Summary Table(s) (6 pages max)**:* As a reminder, state AUT in annual hours, as introduced in the Justification of Need Section. Then, show a table summarizing Research Projects of Users. The table should have the following columns: User's name, grant number (for NIH awards list the grant numbers as R01IC123456), brief title of the project, grant start and end dates, and estimated percentage of AUT hours. If there are multiple Users funded by the same grant, list a total of their estimated percentage of AUT hours of use of the instrument for projects supported by that grant. In addition, make a separate table to indicate the users' needs for any requested accessories. Do not list users whose annual usage is at the level of 1% or less of AUT.

- **Guidelines:** Per ACSB, Two tables should be included. The first table lists the users, their role in the project (major or minor user), title of the project, funding source including grant number, and percent use. Table two lists the users, use and applications, and accessories and features needed. At least three of the major users must need the requested options or accessories to justify their inclusion in the grant request.

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Goldman– Summary Tables

Major Users

D.1. Major Users of this Equipment/Facility

Last name	First name	Department	NIH Grant Number	Project Title	Start Date	End Date	Est. % of Use
Goldman	Yale E.	Physiology	P01-GM087253	Cytoskeletal Motors and Scaffolds in Membrane Dynamics and Motility, Section RT2	9/01/14	7/31/19	12
			R01-GM086352	Structural Dynamics of Actomyosin Motility	1/01/13	12/31/16	
			R01-GM080376	Single Molecule Dynamics of mRNA Translation	8/01/13	05/31/17	
Axelsen	Paul	Pharmacology	R01-GM076201 R01-NS074178	Structure Determination by Vibrational Spectros-copy	6/1/07	5/31/18	8

Minor Users

D.2. Minor Users of this Equipment/Facility

Last name	First name	Department	NIH Grant Number	Project Title	Start Date	End Date	Est. % of Use
Deutsch	Carol	Physiology	R01-GM052302	Biogenesis of Voltage-Gated K+ Channels	5/01/12	2/29/16	3
Hoshi	Toshinori	Physiology	R01-GM57654	Regulation of calcium-activated potassium channels by lipid messengers	9/01/11	05/31/16	3

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Gupta – Summary Tables

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,	1-Apr-	10%

				Strength, and Regulation	2019 to 31-Mar-2024	
		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%

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Litt – Summary Tables

Conservative estimate of AUT: 8,400 hours

Major User	Grant Number	Brief Title	Start-End Dates	Estimated Usage in Annual Hours
Litt, Brian	R01NS099348	Resection to Treat Epilepsy	09/01/16-05/31/21	2500 hours
Kahana, Michael J.	R01MH055687; R01 NS106611; U01 NS113198	How single neurons in the human brain encode space and time	09/15/2019 - 06/30/2024	100 hours
Yoshor, Daniel	R01EY023336	Visual Form Perception Produced by Electrically Stimulating Visual Cortex	09/01/13-01/31/24	100 hours
Bassett, Danielle S.	NSF IIS-1926757	Collaborative Research: Analysis, prediction, and control of synchronized neural activity	09/01/19-08/31/23	60 hours

Lucas, Timothy H.	R01NS113366	The role of dynamical criticality in human perception	06/01/20-05/31/25	120 hours
Gottfried, Jay A.	R01DC018075	Spatiotemporal Mechanisms of Olfactory Processing in the HumanBrain.	04/01/20-03/31/25	60 hours
Davis, Kathryn A.	K23 NS0929731; RFMH117188	Localizing Epileptic Networks Using Novel 7T MRI Glutamate Imaging; From ion channel dynamics to human EEG and MEG: multiscale neuronal models validated by human data	04/01/16-04/01/21; 09/01/18-08/31/22	2500 hours
Cohen, Yale E.	R01DC017690	Coincidence and continuity: uncovering the neural basis of auditory object perception	06/01/19-05/31/24	60 hours
Minor User	**Grant Number**	**Brief Title**	**Start-End Dates**	**Estimated Usage in Annual Hours**
Gold, Josh	Internal Funding and T32 NS091006 (supporting trainee, Dr. Ashwin Ramayya)	Neural mechanisms underlying adaptive decision making in the human brain	07/01/2019-06/30/2021	60 hours
Kelz, Max B.	University Grant	Multi-Sensory Integration: A Neurophysiologic Correlate of Conscious Perception	02/01/17-06/30/20	60 hours

Total estimated usage time by Users' projects in annual hours: 5,620 hours (note the Davis and Litt Labs will utilize and collect all available intracranial EEG data)

Percentage of the estimated usage time devoted to Major Users' NIH-funded projects:

97%Percentage of the estimated usage time devoted to NIH-funded projects: 98%

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Sharp – Summary Tables

Major Users

Table D1. Major Users

Synopsis: 10 of 13 major users are PI's with current NIH funded projects and account for 75% of the total AUT

Major User	Funded projects of major users (From NIH unless indicated otherwise)	Project Dates	AUT (%)
Ronen Marmostein	NIH R35 GM118090-01 (Dr. R. Marmorstein) Molecular Mechanisms and inhibition of Protein Acetyltransferases	06/01/2016-04/31/2021	8
	NIH R21 AI1126317-01 (Dr. R. Marmorstein) Molecular Basis for Activity by Membrane Bound O-Acyltransferases	3/01/2016-2/29/2018	
	NIH P01 CA 114046-07 (Dr. M. Herlyn) Targeted Therapies in Melanoma	05/16/2008 - 08/31/2018	
	NIH P01 AG 031862-07 (Dr. S. Berger) Epigenetics of Aging and Age-Associated Diseases	3/15/2008-10/01/2018	
	NIH P01 CA 025874-33 (Dr. M. Herlyn) Human Melanoma – Etiology, Progression and Therapy	8/25/2011-3/31/2016	
James Shorter	NIH R01GM099836 (PI: Shorter) Defining the mechanistic basis of a prion disaggregase.	01/01/2013-11/30/2016	8

Minor Users

Table D2. Minor Users

(Through the Cryo-EM core, Director Dewight Williams, Ph.D, totalling about 10% of AUT)

Mnor Users	NIH funded projects of minor users	
Benovic, Jeff (Thomas Jefferson Medical Center)	RO1 GM044944	
Cingolani, Gino, (Thomas Jefferson Medical Center)	RO1 GM100888	

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Continue on to Administration (Organizational/Management Plan)

Instructions for Administration **(Organizational/Management Plan) (6 pages max)**: Describe the organizational plan to administer the grant. Describe how the instrument will be utilized, how requests to use the instrument will be made, how time will be allocated among Major Users, how other projects and new

users will be enlisted. Describe how users will be trained in experimental design, instrument operation and data analysis. Describe typical day-by-day management of the instrument.

- **Guidelines:** Per ACSB, This section describes the organization and management plan. The goal is to convince the study panel that the instrument will be well utilized and cared for. The administration section includes:
  - A description of the core facility/entity that will oversee the instrument
  - The location and space where the instrument will reside, including any drawings as needed with any necessary renovations
  - Discussion of the administration of the instrument including the oversight committee, instrument access, scheduling, and dispute resolution
  - Composition and role of technical advisory committee
  - A financial plan including plans for income from charging for use, instrument maintenance, and ongoing support of the service contract. Also note support for the core/technical staff. Provide an operating budget table covering the first four years that includes anticipated expenditures for staff, supplies, and the instrument, usage hours, and anticipated recharge income.
- List the names and titles of the members of the local Advisory Committee. The membership of this Committee should be broad to balance interests of different users and should include members without conflicts of interest (non-users of the requested instrument) who can resolve disputes, if they arise. The membership of this Committee should include at least one senior institutional official who will represent the financial commitment of the institution. Major and other active Users of the instrument may be members, but none may Chair the Advisory Committee. The PD/PI cannot be a voting member of the Advisory Committee.
- The Advisory Committee should meet on a regular basis and should prepare an annual report, which will become part of the Final Progress Report and the Annual Usage Reports (see Section VI.3).
- Describe a plan for managing access to the instrument if users' projects involve human subjects, vertebrate animals or biohazards such as infectious materials.
- Submit a specific financial plan for long-term operation and maintenance of the instrument. Explain how various operational costs will be met; specifically, costs associated with routine operation and maintenance of the instrument, and costs for support personnel. The financial plan *must* include a table for year one of operation with approximate dollars for anticipated expenditures and anticipated income, showing how these estimates were derived. For year one specific dollar amount are required; for years 2 - 5 approximate amounts are recommended.
- Typically, during year one, the maintenance costs are fully covered by one year manufacturer's warranty. In subsequent years, costs of maintenance must be considered in the financial plan. Include a description of projected changes of the financial plan over the subsequent four years.
  - *Operation:* Include salary support of expert personnel that will operate the instrument and oversee routine care and procedures for standardization.
  - *Maintenance:* May include a service contract, or funds for parts and local technical personnel who will maintain the instrument (if such personnel are qualified to do so).
  - *Supplies:* Include necessary supplies for operating the instrument such as chemicals, cryogenics, and other expendable items.
  - *Anticipated Income:* Enumerate the sources of income such as charge back fee structure, grants, or institutional support.

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Goldman - Organizational/Management Plan: As Principal Investigator of this grant and Scientific Director of the Facility, Dr. Goldman has responsibility for day-to-day administrative decisions and coordination. The PI will meet frequently with members of the facility for scientific and/or administrative issues. Business management is the responsibility of the Business Administrator (Ms. Ellen Martin), who ensures that fiscal transactions are in compliance with NIH and University regulations and, with the aid of other departmental business administra-tors, provides the project investigators with up-to-date budgetary information. The University has implemented an integrated campus-wide financial management and budgetary system. The overall business management is directed by the Comptroller of the University of Pennsylvania, and is coordinated through the Director of Office of Research Administration. A full-time PMI Coordinator (Ms. Ashley Douglass) is funded by the School of Medicine and is available to Dr. Goldman for scheduling and correspondence assistance.

E.2. The Scientific Advisory Committee:

Name	Department	Status
Dr. Kevin Fosket	Physiology (Chairman) School of Medi-cine (SoM)	Chair of the Advisory Committee
Dr. Elizabeth Rhoades	Chemistry, Faculty of Arts and Sciences	Member of Committee
Dr. Louis Soslowsky	Orthopedics, SoM, Associate Dean for Core Facilities	Member of Committee
Dr. Sergei Vinogradov	Biochemistry and Biophysics, SoM	Member of Committee
Dr. Yale E. Goldman	Physiology, SoM	PI, Ex-Officio Member of Committee
Dr. Paul H. Axelsen	Pharmacology, SoM	Co-PI, Ex-Officio Member of Committee

The PI/Director, Dr. Goldman, and Scientific Advisory Committee will meet yearly to discuss access usage policies and fees. For usage, calendar software, called phpScheduleIt, is maintained on School of Medicine servers by the School's IT staff and can be accessed any time by users via web browser. The Director controls access to the sign-up calendar, so only users who have received training from facility staff are able to reserve equipment. This is managed on a daily level by the Technical Director of the Facility, Dr. Pyrpassopoulos. The reservation software allows for straightforward statistical analysis of usage and also provides the basis for bill-ing. Monthly use is tracked by the Technical Director, and users are provided with documentation prior to debit-ing of accounts.

The estimated budget for establishing the MFD-FRET facility in FY16-17 is given below. The initial start-up costs include equipment and installation of the MicroTime 200 and offline analysis workstations. Costs for Dr. Pyrpassopoulos's effort and service contracts after the year included with the purchase will be covered by the School of Medicine during the first five years of operation to the extent that they are not offset by user fees (please see Dr. Epstein's letter). Consumables will be purchased by users through their research grants.

E.3. Measures to Assess Quality of Service

A survey will be sent our each year to users and prospective users to assess satisfaction, quality of service and suggestions. The staff will make constant effort to interact with investigators to design protocols for each new project, to select the most reliable and efficient technique to obtain fluorescent labeling of target molecules and obtain results in the shortest time, and to interpret MFD-FRET data.

E.4. Projected Budget for Purchases:

Quantity	Item	Cost per Unit	Extended Cost
1	PicoQuant MicroTime 200	$494,696	$494,696
2	Off-line SymphoTime 64 Analysis Software	7,084	14,168
2	Dell PC Workstations and Monitors	5,292	10,584
	Total Cost of Purchases (Current S10 application)		$519,448

To the extent possible, usage fees will be set by the Director and Advisory Committee to offset ongoing costs for maintaining and operating the equipment, as well as a portion of the salary for the Technical Director and other staff. Based on feedback from the faculty of reasonable fees and experiences from our other cores, we will initiate the following cost recovery:

A) Initial consulting and advice no charges will accrue. A one-day feasibility study fee will also be waived for those users seeking preliminary data for NIH grant applications

B) Upon initiation of a project with the facility, all consultations to review a project's design, status or data will be charged at a rate of ~$50.00 per hr. Staff and equipment will be available for training, protocol development and optimization. This is priced hourly depending on equipment and labor needs. Once trained, the instrument and analysis software may be operated by users without oversight.

We will charge all users the same rate, regardless of whether or not they are major or minor users. We will estimate future usage fees based upon service contract rates and technical consulting/actual experimental effort by the facility manger. The anticipated operation costs and user fees (from experience of other core facilities) are listed below. We appreciate that even after Year-3 it may not be possible to fully recover the costs of operation, and the School of Medicine has committed to underwrite such expenses for the expected life of the instrument (please see Dr. Epstein's letter of support). Hence, it is not necessary for user fees to entirely cover all costs of maintenance and operation, although it is recognized that revenues will offset institutional costs and allow for additional new initiatives and upgrades. Institutional Commitment

E.5. Three year Estimate of Facility Operating costs

Item	Year 1	Year 2	Year 3
Salary and EBs for Facility Manager	$50,000	51,500	53,045
Equipment Service Costs	0	50,000	50,000
Supplies to be supplied by user grants	0	0	0
User Fees	(30,000)	(60,000)	(60,000)
	$20,000	$41,500	$43,045

Importantly, the School of Medicine is fully committed to all of the startup, operation and maintenance costs for years 1-3, and if necessary beyond, so that the facility may build full competency and establish its user

base. Once steady state is achieved, we expect major users to account for approximately 50-60% of total usage for the PicoQuant MicroTime 200 microscope and they will have priority in reserving microscope time. Any disputes in usage will be resolved by the Advisory Committee. As a key component of our facility, the instrument will also be available to researchers outside the major user group, who will be charged at the rates de-scribed above. As indicated, 12 users have expressed a strong interest in utilizing the MFD-FRET microscope, fluorescent labeling and computing assistance provided. Lastly, as the publications listed by our prospective users indicate, we have demonstrated the strong need for structural dynamics in isolated macromolecules and hence the importance of this equipment request.

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Gupta – 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.

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Litt – Administration (Organizational/Management Plan)

The Blackrock Neuroport system will be housed in Penn's new state-of-the-art patient care Pavilion that supports clinicians, researchers and faculty. The equipment will be conveniently located in designated research space that is contained within the Department of Neurology's clinical care area, where the 12-bed Epilepsy Monitoring Unit resides. Investigators will easily be able to move the equipment from the assigned space to the patient's room which has been designed to accommodate research equipment and personnel.

As Director of the Epilepsy Monitoring Unit (EMU), Dr. Kathryn Davis oversees all activities taking place in the in the Unit, including all IRB approved research testing. Dr. Davis provides oversight for a well-functioning system already in place for research scheduling and testing. An Administrative Coordinator in the Center for Neuroengineering and Therapeutics manages the testing calendar and is in regular communication with the EMU staff to ensure that the patient's clinical care and needs are being met prior to initiating research testing. Investigators are able to prioritize their research based on the electrode configuration and the patient's willingness to be tested. Additionally, Dr. Davis supervises a well-attended monthly meeting that is held for investigators and their research teams. This provides an opportunity for investigators to present their research, discuss testing logistics and is a forum for promoting collaborative research utilizing the Blackrock system. This regularly scheduled meeting also serves as a time to remind investigators to cite the S10 award in their publications.

All individuals named in the proposal have extensive experience in utilizing neural recording systems and analysis of the acquired data. For users unfamiliar with the Blackrock system, a formal training session will be scheduled and hands on support will be provided until the investigator is able to independently run their own experiments. The team will also take advantage of Blackrock learning resources, such as workshops, and training as needed. The Penn neuroscience community is expanding and is committed to mentoring junior faculty and minor users in this collaborative translational research space. In addition to fostering collaborative research within the Perelman School of Medicine, investigators on this project are continuously seeking out collaborative projects with the many talented researchers in the Vet, Dental and Nursing Schools at Penn, as well as its neighbor the Children's Hospital of Philadelphia.

Advisory Committee:

The Advisory Committee is composed of the following Clinician Scientists and Dr. Soslowsky, Associate Dean for Research Integration, multiple of which are nationally recognized for their expertise in neuroscience and translational medicine.

Frances Jensen, MD, FACP

Professor of Neurology
Chair Department of Neurology
Co-Director, Penn Medicine Translational Neuroscience Center

Daniel Yoshor, MD

Professor of Neurosugery
Chair Department of Neurosurgery
Vice President of Clinical Integration and Innovation for the Health System

Louis Soslowsky, PhD

Fairhill Professor of Orthopaedic Surgery

Professor of Bioengineering
Associate Dean for Research Integration
Vice Chair for Research – Orthopaedic Surgery Director of
Penn Center for Musculoskeletal Disorders

Kathryn Davis, MD, MSTR, FAES

Assistant Professor of Neurology
Epilepsy Monitoring Unit Medical Director, University of Pennsylvania

Epilepsy Surgical Program Medical Director, University of Pennsylvania
Associate Director, Center for Neuroengineering and Therapeutics, University of Pennsylvania
Assistant Director of the Penn Epilepsy Center, University of Pennsylvania

Timothy Lucas, MD, PhD
Assistant Professor of Neurosurgery
Surgical Director, Penn Epilepsy Center, University of Pennsylvania

Colin Ellis, MD

Assistant Professor of Neurology

Dr. Frances Jensen has agreed to Chair the Advisory Committee and as an expert in brain development throughout the lifespan and Co-Director of the Penn Medicine Translational Neuroscience Center she is highly qualified to guide the committee and all of the Investigators on the grant. The committee will meet biannually and will prepare an annual report which will become part of the Final Progress Report and Annual Usage Reports. The Committee will also oversee compliance of citing the S10 award in their publications and will determine if compliance has been met. As Chair of the committee, Dr. Jensen will also represent the financial commitment of the University, ensuring that investigators have the resources they need to fully utilize the Blackrock Microsystem. Dr. Ellis will serve as an epilepsy clinician not directly involved in the research but with expertise in the clinical management of intracranial EEG patients. Dr. Soslowsky, Associate Dean for Research Integration, will serve on the committee and assist with ensuring that the BlackRock is successfully integrated into the multiple research programs as proposed.

<u>Financial Plan:</u>

All investigators named in this proposal are familiar with the use and routine maintenance of neural recording systems. Penn received an S10 grant for a 256 channel Neuralynx in 2011 and has successfully operated and maintained the equipment which is still in use. Dr. Yosher and his research team have expertise in using and maintaining a BlackRock system at Baylor and will bring this experience to Penn, taking the lead on guiding use of the equipment, particularly during year one. Dr. Yoshor is also a memberof the Advisory Committee and is prepared to take an active role in ensuring the successful collaborative utilization of the BlackRock system at Penn for translational research.

Operation: The BlackRock system will be housed in designated research space in Penn's new state-of-the-art patient care Pavilion. This space is located within the Neurology patient care areaand all investigators will be provided access to the equipment. Individual PIs will support the trained research personnel who will operate the instrument on their funded research grants in all5 years of the grant

Maintenance: Prior experience at Penn with a Neuralynx system and experience at Baylor with a BlackRock system indicate that routine maintenance for the equipment and software can be successfully be carried out by trained research staff for the life of the recording system. Warranty service from the manufacturer will cover the cost of any service that is required in the case of a malfunctioning part.

Supplies: The cost for clinical electrodes being recorded with the BlackRock system are bundled into charges for clinical care. Individual Investigators will support the cost for research electrodes.

Software: The Penn BlackRock Neuroport system will include a complete software package with networking capability and software development kits for MATLAB and C++. Penn has a MATLABsite license and Penn IT staff support the software installation. The cost for the yearly software update is $150 and will be supported through funded research grants. C++ is a programming language that is virtually open source and is very familiar to the investigators and their research teams. There are no expected expenses related to the use of C++. Users who require specialized software for their data analysis will fund this through their research grants.

Anticipated Income: There is no expected income from the use of the BlackRock neural recording system for research purposes.

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Sharp – Administration (Organizational/Management Plan)

E. Administration

E.1. Local Advisory Board

Name	Titles/Positions
Jason H. Moore, Ph.D (Board Chair)	Director, Institute for Biomedical Informatics, Director, Division of Informatics, Senior Associate Dean for Informatics
Lyle H. Ungar	Professor of Computer and Information Science, former Associate Director of the Penn Center for Bioinformatics.
Mitchell Lewis, D.Phil	John Morgan Professor of Biomedical Research and Education, Interim co-Chair, Dept. of Biochemistry and Biophysics

The Advisory Board consists of senior and distinguished scientists, non-users of the requested resource, who have broad experience with biomedical computing within PSOM. The Board Chair is a senior institutional official holding the position of Senior Associate Dean for Informatics. The other two Board members have extensive prior computational experience. The Advisory Board, along with the PI Dr. Sharp as a non-voting member, will set and achieve strategic goals for the resource, advise on usage and maintenance of the resource, and help maximize the support it will provide for the NIH funded users in Structural Biology and Molecular Biophysics.

E.2. Operational Board

Name	Role
Kim Sharp Ph.D, Dept. of Biochemistry and Biophysics	PI, administrator of computer equipment.
Paul Axelsen, Dept. of Pharmacology	Applications and technical support
Dewight Williams, Ph.D EMRL core director	Applications and technical support

The Operational Board will implement the day-to-day operations of the resource. Dr. Sharp will handle the systems administration, installation and upgrades of software, with technical advice on software applications from Dr. Axlesen and Dr. Williams in their areas of expertise.

E.3. Scheduling

The requested instrument is computer hardware. Based on experience with our two previous clusters, we budget about 1 day per month down time (system unavailable to users) for system maintenance, operating system software upgrades. Aside from any additional downtime due to hardware replacements, available user time is essentially 24hrs per day, 7 days a week, amounting to more than 8,000hrs of available user time

(AUT) per year. The requested hardware comes with the Bright Cluster Manager software to manage the cluster (See equipment quote). This software is developed by Bright Computing (www.brightcomputing.com/) specifically to manage clusters containing Nvidia GPU's. It is a comprehensive management software for provisioning, monitoring, managing and job scheduling. Given the nature of 'dense' computing, and the choice of new hardware, the planned mode of use is for each user to be given total use of the cluster for a single job. Simultaneous multi-job use would defeat the design. With this sequential mode of use, job scheduling and efficient use of the cluster through Bright Cluster Manager is straightforward.

E.4. Storage

In addition to the approximately 400Tb of storage available with the initial instrument purchase (see equipment quote), the plan is for users to provide additional storage for their own data: The most efficient way to put data on and take it off is through hot swappable external multi-Tb capacity disk drives. The requested hardware has this capability. These drives currently cost about $20 per terabyte, and would represent a modest expense for the major users.

E 5. Financial plan of Operation

Computer resource costs are almost entirely in capital, in the initial purchase. The major operational costs are power, cooling and administration, which will be covered by the school of medicine as part of their commitment to the requested instrumentation. With electronic data transfer and storage, there are no supply expenses to speak of. Thus the cost to users for using the requested instrument will amount to providing the extra storage they need to manage the data for their specific projects.

Table E.1 Operation in Year One

Table E.1. Operation in Year One

Item	Source/Costs per year
Power and Cooling	Provided by the B&B Dept. (please see Dept. letter of support)
Operation (System Administration)	Salary and benefits equivalent to 2.0 cal month effort for the PI, provided by the Biochemistry and Biophysics Dept. (please see Dept. letter of support)
Additional Storage	Provided by Major Users as needed. See section E.4.
Hardware Replacements or upgrades (Beyond manufacturer's 3yr. warranty)	Up to $10,000 per year as needed. Provided by the Biochemistry and Biophysics Dept. (please see Dept. letter of support)
Income	None

The financial plan for second and subsequent years of operation through the lifetime of the equipment will be essentially identical to that in Table E.1.

The financial plan for second and subsequent years of operation through the lifetime of the equipment will be essentially identical to that in Table E.1.

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Instructions on Institutional Commitment (3 pages max): Describe the institutional infrastructure available to support the instrumentation, including space to house the instrument and site for sample preparation, if applicable.

- **Guidelines:** Per ACSB, State the institutional support. If applicable, it is extremely helpful to include a letter of support from your chair or dean that commits to support in perpetuity (or at least 3-5 years) of the service contract for the requested instrument (with a statement as to how the cost will be covered following the time period – such as recharge). This letter should also include a commitment to cover the cost of any renovations and anything else needed.
  - Confirm the institutional support toward the maintenance and operation of the instrument. In particular, confirm that the institution will commit to provide backup of the financial plan for five

years from installation of the instrument or for its effective/usable lifetime. The expected usable lifetime depends on the type of requested instrument. Describe institutional support for personnel.

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Goldman – Institutional Commitments

F. Institutional Commitments

Encompassing an integrated School of Medicine and Health System, Penn Medicine is America's oldest medical school and an internationally recognized leader in the creation of new knowledge and therapies to improve human health, and in the training of the next generation of scientific leaders. Basic science research is of fundamental importance to the mission of Penn Medicine, and the School is committed to ensuring that we are at the forefront of new developments and innovation in biomedical research.

Newly renovated space on the 6th floor of the Clinical Research Building is available to house the MFD-FRET microscope. This is adjacent to several of the major users' laboratories and is appropriate to the electrical, mechanical and ambient audio requirements of the requested instrument. Nearby biochemistry and macromolecule characterization facilities and a PMI/Physiology conference room are readily available.

We have included letters confirming support from:

1. Executive Vice Dean and Chief Scientific Officer of the School of Medicine, Dr. Jon Epstein

2. Dr. Elizabeth Rhoades, Department of Chemistry and experienced user of MFD-FRET technology

3. Dr. Kevin Foskett, Chairman, Department of Physiology, School of Medicine, Chair of MFD-FRET Facility Advisory Group

4. Dr. E. Michael Ostap, major user and Director of the Pennsylvania Muscle Institute, which will support a training trip to Germany for the facility manager

5. Dr. Claus Seidel, Professor at Molekulare Physikalische Chemie: Universität Düsseldorf, a main developer of MFD-FRET technology and originator of much analytical software available to us

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

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Gupta – 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.

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Litt – Institutional Commitments

The University of Pennsylvania has contributed start-up funds and continued support to our collaborative Neuroengineering Group and the Penn Epilepsy Center. Among these includes a brand new twelve-bed EMU in our new state-of-the-art hospital, to open in 2021. In addition to equipping the EMU for advanced tertiary care and epilepsy surgical evaluation, Penn has devoted over 400 square feet for a research room to support the team's collaborative efforts and linked this room with high bandwidth fiber optic internet and high bandwidth wireless internet through dedicated conduit to each of the EMU rooms. In November 2013, the Deans of the Schools of Medicine and Engineering charged Dr. Litt with founding a new Center for Neuroengineering and Therapeutics (CNT) to bridge their two schools in an exciting multidisciplinary effort to formalize Penn's incredibly rich and productive tradition of excellence and innovation in this space. The schools together committed resources in excess of $6 million to Dr. Litt to implement this vision, which is to be dispersed evenly over 10 years. Dr. Litt's Center for Neuroengineering and Therapeutics, has proven to be the perfect setting for this kind of program, and he and Dr. Kathryn Davis have assembled a collaborative group of investigators who work together on translational research using our epilepsy inpatients implanted with intracranial electrodes. This group has been tremendously productive and the additional of the BlackRock system will enable substantial scientific advancement in cognitive neuroscience and epilepsy.

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Sharp – Institutional Commitments

F. Institutional Commitment

Included are strong letters of support from:

1. The co-Chairs of the Biochemistry and Molecule Biophysics Dept.

2. The Senior Associate Dean for Informatics and Chair of Advisory Board.

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Continue on to Overall Benefit

Instructions for Overall Benefit (3 pages max)*:* Explain how the instrument will impact NIH-funded research and contribute to the institution's long-range biomedical research goals.

- **Guidelines:** Per ACSB: State in one or two paragraphs the broad benefit of the new instrument to the greater research community. It is fine to place the instrument in the context of the core facility and communicate the instrument's broad benefit to the core facility and to the research infrastructure of the university.

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Goldman – Overall Benefit

G. Overall Benefit

The aim of this proposal is to purchase a state-of-the-art novel microscopy workstation for advanced structural dynamics investigation not feasible by other means. As Penn investigators, and especially the major and minor users of this facility, are leaders in biomedical research, centralized access to this novel technology will foster the most advanced biomedical, health-care related research. The facility will initially support the research activity of 6 Major users and 6 Minor users who are NIH-funded (>$3M) in three departments within the School of Medicine. The facility will provide:

1. Advice, training, protocols, and/or physical biochemistry to place reporter probes in specific and appropriate sites on target research protein and nucleic acid macromolecules and small molecule ligands.

2. Training and access to a novel state-of-the-art microscopy workstation for structural dynamics and highly reliable MFD-FRET efficiency, occupancy and dynamics measurements.

3. Advice, training, and/or conduct of pre-programmed analysis software and/or molecular dynamics after-experiment processing of data to convert FRET efficiency dtat into distance and kinetic information.

4. The facility will generate revenue to pay for the service contracts and salaries and maintain its mission long term.

The request in this application represents essential equipment for examination by single molecule fluorescence energy transfer research-related biological material. Rather than an upgrade to existing, available equipment, it is a novel and unique research tool available in only a few laboratories around the world and not currently available to UPenn investigators. The research that will benefit from this instrumentation promises to contribute to the translation of basic biomedical research to treatments and cures. This instrumentation will benefit research to understand basic biological systems and develop important new therapeutic strategies against numerous disease states, including cancers, stroke, degenerative neurological disease, heart disease, orthopedic conditions, and thrombosis and bleeding conditions. As outlined in this application, acquisition of this equipment will make this technology available to a large number of investigators, in keeping with the goals of the school and the national biomedical research effort.

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Gupta – 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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Litt – Overall Benefit

As noted above, this project is one that is the product of explosive growth in our collaborative and translational research between the Schools of Medicine and Engineering at Penn, as well as a large effortto collect the highest quality human intracranial electrophysiology and share it world-wide through the International Epilepsy Electrophysiology Database, funded through the NINDS. Central to this continued growth is the newly established 12 bed epilepsy monitoring unite (EMU) in the Pavilion hospital, which contains a dedicated research space specifically for human neurophysiology experiments. Furthermore, allowing the purchase of this equipment will not only skyrocket our productivity in collaborative human research, but it will enrich our ~15 existing collaborations with other major epilepsy centers across the United States and worldwide including the Mayo Clinic, New York University, and University of Melbourne, and greatly enhance our training of students in Neurology, Neurosurgery, Neuroscience, and Engineering. For a relatively modest investment we anticipate a huge payoff.

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Sharp – Overall Benefit

G. Overall Benefit

The computer equipment requested in this proposal represents state of the art hardware for algorithmically dense computing, specifically chosen for the computing needs of the Structural Biology and Molecular Biophysics community of researchers, primarily, but not exclusively located in the Perelman School of Medicine. It is designed to leverage existing instrumentation and research groups working in the areas of Cryo-Electron Microscopy, NMR, X-ray crystallography and Scattering, Single Molecule Biophysics, High Resolution Mass Spectroscopy (MS), Hydrogen Exchange (HX) and Macromolecular Simulations. It will enable this existing instrumentation to be used to its full potential. It will also remove accumulating barriers to major research projects impacting 26 currently funded NIH projects, representing more than $6,700,000 of research support per year. In addition it will aid 6 more projects funded by other federal and private sources.

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Continue on to Letters of Support and Other Attachments

***Instructions for Letters of Support and Other Attachments**:* All letters of support should be combined in a single file named Letters of Support and uploaded as a separate attachment via Other Attachments. This combined file should include, as applicable:

- Letters from institutional officials;
- Institutional back-up for the proposed financial plan;
- Letters about inventory of instruments at the institution which are unavailable to the PD/PI (as noted in the Justification of Need Section).

- The institution must also provide a table that includes information about performance of all previous S10-awarded instruments within the past five years; that is, FY 2011 - 2016. The table should have the following columns:

    1. S10 Grant Number
    2. Year of Award
    3. Installation Date of the Instrument
    4. PD/PI's name; Generic Name of Instrument
    5. Instrument Status: (Active (instrument in use), Pending (order placed, but instrument not delivered, instrument received but not installed or not calibrated for general use), Upgraded (or replaced), Not Available (sold, decommissioned, transferred)
    6. Actual Usage Time (actual total time in hours per year the instrument was used for research; if the instrument has been installed less than a year ago, the hours can be extrapolated for an estimate of hours per full year)
    7. Maintenance Agreement (Active (Warranty in place), In-House (or Self-Insured), None (Fee for Service, Pending), Not Available (no longer supported by manufacturer)
    8. Number of Publications Citing the S10 Award

- If the instrument is currently non-functional, the institution must provide a supplementary explanatory text.

- If human, animal, or infectious materials, which could create a potential biohazard, are to be analyzed, a signed letter from the institutional biosafety officer stating the proposed containment plan was reviewed and adheres to documented biosafety regulations. If relevant, this letter is required in the application.
- Biosketches: Include biosketches (in the standard NIH format) of Major Users, Minor Users, and technical personnel, as applicable. Biosketches don't count towards the page limitation.

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