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. Lou Soslowsky (soslowsk@upenn.edu)

This document contains guidance for the NIH Shared Instrumentation Grant (SIG) PAR-18-600. 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!

Purdue University:
Self-Help Tools for Proposal Preparation – Preparing Major or Shared Research Instrumentation Proposals

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 PAR-18-600 as this is the official NIH document on this grant opportunity.

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: Cherry, Goldman, Sharp)
- Technical Expertise: 3 pages in total (Examples: Cherry, Goldman, 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: Cherry, Goldman, Sharp)
- Summary Table(s): 6 pages in total (Examples: Cherry, Goldman, Sharp)
- Administration (Organizational / Management Plan): 6 pages in total (Examples: Cherry, Goldman, Sharp)
- Institutional Commitment: 3 pages in total (Examples: Cherry, Goldman, Sharp)
- Overall Benefit: 3 pages in total (Examples: Cherry, Goldman, 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 – 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 oversight 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 be-tween 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:

1. 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 con-figuration 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 $\kappa 2$. 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.

Return to Section Headings Index
Return to Section Instructions – Introduction to Resubmission
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.

Return to Section Headings Index
Return to Section Instructions – Justification of Need
Return to Section Guidelines – Justification of Need
Continue on to Justification of Need - Cherry Example

Cherry - Justification of Need

A1. The Penn Screening Core.

Following a ground-up process, driven by faculty at Penn, the Executive Vice Dean/CSO charged Dr. Morris Birnbaum, Assistant Dean for Core Facilities, to analyze the need and requirements for a new high throughput Screening Core. This process identified over 30 Penn faculty who self-declared as potential users. Nineteen of these faulty are ultimately identified in this proposal as either major or minor Core users. The combined NIH funding of these investigators is $21M in FY14. Currently, there is no Screening Core that is available to these investigators, or others who may emerge over time. This is thus a major unmet need at Penn.

The capability to perform high-throughput screening as a key component of assessing molecular Screening (HTS) requires the miniaturization, optimization and automation of bioassays so that millions of variables can be tested and is often an important step in the discovery of new medicines and therapeutic avenues. This Core will assist Penn faculty in optimizing assays to allow for high throughput screens of chemical and genetic libraries to identify new tools and targets for biological research. The Core is built around modular work stations with the **Janus Varispan + Modular Dispensing Tool (MDT) Workstation** at the heart of the Core. This Core/equipment will aid researchers in improving the efficiency and potency of their bioassays, ultimately with the goal of identifying compounds and genetic modulators of function. This will reveal new insights into biology, generate new biological tool compounds, provide proof-of concept for larger-scale screening (e.g., large compound libraries at NIH) and will directly advance translational medicine at Penn.

The Core will facilitate screening of two types of perturbagens (small molecule and genetic) in either biochemical or cellular assays. Assay development and optimization is the key, and rate-limiting step for most screening. This will be the major focus of the core. Once assays are developed using small scale libraries, larger screens can be performed or the small scale libraries may be the end point for discovery. We will have focused libraries of small molecules including FDA and FDA-like drugs that can be used for phenotyping of diverse cell types or complex biologies. The Core will also support genetic screening for loss-of-function analysis using siRNAs with most investigators focused on sub-libraries (eg kinome) active against human and mouse genomes. miRNAs have emerged as key regulators in vivo, and thus we will house collections of human and mouse mimetic and inhibitor miRNA libraries (small libraries).These will be complemented with a cDNA library for gain-of-function screens (MGC Collection) and genome-wide collections.

For assay development and screening, the Core will be equipped for processing microtiter plates through the use of liquid handling and imagers for standard well-based and microscopy-based assays. This includes absorbance, fluorescent kinetics, fluorescence anisotropy, time-resolved fluorescence, time-resolved fluorescence resonance energy transfer, AlphaScreen, bioluminescence and automated microscopy. Assay targets can include ion channels, receptors, enzymes, protein interactions, signaling pathways and
cellular processes. We will facilitate the development of a robust assay that will be tested in pilot screens. Once these pass statistical criteria then larger screening can commence. Upon completion of the screening, validation of the candidates will be performed.

A2. Personnel of the Core.

Dr. Sara Cherry, an Associate Professor in the Department of Microbiology who was jointly hired into the Penn Genome Frontiers Institute is a leader in high-throughput screening. She trained with Dr. Peter Schultz as an undergraduate, Dr. David Baltimore as a graduate student, and Dr. Norbert Perrimon as a postdoctoral fellow. She has >10yrs of experience performing HTS screens using small molecules and genetic perturbants. She has also performed both well-based (luminescence) and image-based screens. She has collaborated with a large number of Investigators to perform screens across diverse biologies. Within her own laboratory she has provided infrastructure, libraries and expertise to perform HTS screens for >10 investigators since her appointment at Penn in 2006. More recently, linked to funding from the Mid Atlantic Regional Center of Excellence she provided assistance to screen viral pathogens for several investigators within this Center. This

resulted in four genome-wide RNAi screens performed by Drs. Ross, Doms and Isaacs at Penn and Dr. Judith White at UVA, resulting in a number of publications in high impact journals (e.g., Cell, Genes & Dev, Science Trans. Med). Perhaps more importantly, the identification of potential therapeutic targets of alphavirus, bunyavirus and arenavirus infection were also identified. This highlights not only the unmet need, but the ability of such endeavors to be externally supported. And therefore, Dr. Cherry is well-suited to Direct this Core and oversee its operation.

Technical Director: The proposed facility will be managed by a Technical Director, to be hired, who will have HTS experience. The Technical Director will manage a staff of additional key personnel and provide individualized consultation to PENN faculty on high-throughput screening projects. The Technical Director will be responsible for the day-to-day operation of the Core, including all aspects of project management including, assay development, compound management, HTS, data analysis, and post-screen validation studies. All facility personnel will report to the Technical Director.

Staff scientist: A Ph. D. level staff scientist will ultimately be hired, linked to demand, to perform optimizations, high-throughput screening, and post-screen validation studies. They will also help with day to day operations. The salary of Dr. Cherry is supported by external grants and institutional funding while the salary of the Technical Director and staff scientist will be recovered from user fees generated by the Core, and supplemented by institutional funds.

Oversight: An internal and external Advisory Committee will be assembled to develop strategic plans that meet the scientific needs of Penn. The internal Advisory Committee will be chaired by Dr. Morris Birnbaum in his capacity as Assistant Dean (see Administration section below).

The centralization of these services in a single Core laboratory with an experienced staff in all areas will allow for timely project management, quality assurance, and dissemination/integration of data critical for the translation of basic biological observations into potential therapeutic strategies and promote intra- and inter-programmatic collaborations critical to the mission of Penn to improve human health.

A3. Equipment in the Core.

The Screening Core will provide scientists with state-of-the-art HTS services of small molecule, siRNA, miRNA, shRNA, and cDNA libraries to identify genes that impact the biology of interest, small molecule profiling of cell types of interest, and new tool inhibitors of candidate therapeutic targets. These will promote new discoveries and, in addition, the identification of drug-like, small molecules that regulate the activity of newly validated therapeutic targets holds great promise to define new treatment paradigms with better efficacy and therapeutic index for recalcitrant disease, where current clinical practices are largely ineffective. This may be most rapidly realized using FDA or FDA-like compound screens.

More specifically - the core will: (1) develop biochemical-, cell-, and high-content based assays amenable to high-throughput screening in 96- and 384-well microtiter plates that demonstrate a reproducible and sensitive response to control conditions; (2) manage libraries of small molecules, siRNA, shRNA, cDNA and miRNAs; (3) execute high-throughput screening experiments of small molecule and genetic libraries; (4) analyze biological and chemistry data sets; (4) characterize the potency and selectivity of newly identified compounds in secondary, orthogonal assays; and (5) provide educational Workshops for the community to aid in assay design, optimization and screening library choice. The Core will be equipped with laboratory robotics, libraries of siRNAs, shRNAs, miRNAs, cDNAs and drug-like molecules arrayed in high-density micro-plate formats. **The Janus Varispan + Modular Dispensing Tool (MDT) Workstation** lies at the heart of the Core. The Core will also be linked to computational infrastructure for efficient analysis, interpretation, and management of biological and chemistry data sets in concert with the Penn Institute for Biomedical Informatics.

Equipment

The following equipment will be included in the Screening Core Facility at Penn.

Automated liquid handling/pipetting workstations: **The Perkin Elmer Janus Modular Dispensing Tool (MDT),** automated liquid pipetting workstation for compound/reagent transfer and library management (requested herein). The Janus MDT is equipped with a standard P30 384 well head capable of transferring volumes precisely between 0.5 ul to 25µl with disposable tips. A fixed tip, nanoHead dispense tool enables precise delivery of volumes as low as 50 nl to assay plates. The P235 96 well head enables the pipetting of volumes between 20 to 235 ul, using disposable tips. The Janus MDT workstation is integrated with a plate stack unit with a maximum capacity for 50 microplates.

A **Perkin Elmer Janus Verispan 8 channel pipetting** workstation for use in reformatting libraries (e.g., dilution series, Hit-Picking) and inserting controls on assay plates (requested herein). The Verispan 8-tip is designed for use with either fixed washable tips or disposable tips in 20 ul, 200 ul, and 1 ml sizes, and disposable filter tips in 25 ul and 175 ul sizes.

For bulk reagent dispensing, Well Mates can be used. A Biotek ELx405 automated plate washer with a 96 channel head that can be used with 96- and 384-well plates. This plate washer enables the facility to support ELISA and cell based staining experiments where excess material needs to be effectively removed prior to reading. The Elx405 offers the flexibility of integration with the plate stacker, as necessary, to automate procedures for higher throughput.

Envision Microplate reader: The Molecular Screening Facility will also include a PerkinElmer EnVision Xcite Multilabel plate reader to read 96- , 384-, or 1536-well assay plates. The EnVision Xcite plate reader is capable of standard multi-mode detection, including Absorbance, Fluorescence Intensity (FI), Luminescence, Ultra Luminescence, Time-Resolved Fluorescence, Fluorescence Polarization (FP), AlphaScreen (Amplified Luminescence Proximity Homogeneous Assay). The reader is equipped with a wide selection of filters and dichroic mirrors for most standard dyes and dye-pairs and a plate stack.

High-Content Screening Reader: The ImageXpressMicro is an inverted epifluoresence microscope that utilizes a laser auto-focus option to automate acquisition of high-content images in 4 channels from 96- and 384-well microtiter plates or slides. It is equipped with a xenon arc lamp excitation source, a motorized objective wheel with bar-coded, interchangeable objectives (2X, 4X, 10X, 20X, 40X), 5 filter cubes, and a 1.3 megapixel, 14-bit Peltier cooled CCD camera. Image analysis is performed using MetaXpress.

Tissue Culture: Two 6 foot tissue culture hoods, which can house bulk reagent dispensors (e.g. WellMate) to plate cells in assay plates. In addition 4 standard CO2 regulated tissue culture incubators, a benchtop centrifuge, and an inverted phase-contrast microscope for use while passaging and preparing cells for screening experiments.

Data preservation: A major demand associated with high-throughput screening is preservation/archival of data. PMACS will handle the storage of data.

Storage: -80 Freezers, 2 initially to be expanded as needed.

The following Libraries will be available within the Core:

Chemical Libraries: We will begin our collection with focused libraries of bioactives, FDA approved and FDA like compounds.

- LOPAC1280™ (1280 compounds)The LOPAC 1280 collection is a useful library for HTS validation with proven pharmacologically-active compounds.

- MicroSource Spectrum Collection (2,000 compounds)Contains 3 sources: a US drug collection of 1040 drugs that have reached clinical trial stages in the USA, an international drug collection of 240 drugs that are marketed in Europe and/or Asia but have not been introduced in the US, and a natural products collection of 800 compounds, which is a collection of pure natural products and their derivatives.
- Pharmakon (900 compounds)It is a unique collection of known drugs from US and International Pharmacopeia. All compounds within the PHARMAKON Collection have reached clinical evaluation and not simply demonstrated biological activity.
- The Prestwick Chemical Library (1120 compounds). 90% of this collection are marketed drugs and 10% are bioactive alkaloids or related substances.

Genetic Libraries. The core will offer the following libraries for cell-based HTS:
- Human and mouse annotated genome (Silencer Select siRNA library from Life Technologies)
- Human and mouse mimic and inhibitor miRNA (mirVana libraries from Life Technologies)
- TRC shRNA library: The complete (human and mouse) TRC1.0 shRNA library will be maintained as bacterial glycerol stocks. The human library (TRC-Hs1.0) targets 15,000 annotated human genes and consists of 80,700 precloned constructs. The mouse library (TRC-Mm1.0) targets 15,000 annotated mouse genes and consists of 76,800 precloned constructs.
- cDNA library: the Mammalian Gene Collection (MGC) collection that we have contains 18,000 full length, fully sequenced cDNAs from human and mouse driven by a CMV promoter. We have the bacterial glycerol stocks in hand and these will be prepped and arrayed for screening.

A4. Usage of the Core.
We are developing this Core for all Penn Faculty and labs. Researchers who currently have no access to High-throughput Screening will now have access. As noted above, over 30 NIH-funded investigators initially self-identified as potential users. Nineteen of these have contributed to this SIG proposal as either major or minor users. The current major users come from the departments of Microbiology, Cell and Developmental Biology, Medicine and Physiology. Identified minor users additionally encompass Cancer Biology, Pharmacology, Biochemistry, Pathology and Neuroscience. We anticipate that the users will fall into two categories: (1) Those who will go through training and then continue to use the instruments including the Perkin-Elmer Janus Workstation independently for their research; and (2) those who develop assays in their laboratories and have the Core run all of the equipment and assays. Indeed, it is the latter category that will likely make up the bulk of the usage. This Janus Workstation is automated and modular so that if we need additional capacity that can be engineered into the current request.

A5. Other Liquid handling workstations on campus.
The Penn School of Medicine is a large institution, with over $600M in annual sponsored research funding, not including the Hospital of the University of Pennsylvania (HUP) and the Children's Hospital of Pennsylvania. For an institution of its size, it is remarkable that there are no current Screening Core facilities available on a fee-for-service basis to the general research community, and there are surprisingly few liquid handling robots on Campus at all. The list below includes all automated liquid handlers on campus, but importantly these are restricted to individual labs and are <u>NOT available for outside use</u>. In addition, many of these are older models lacking many of the capabilities of the current request.

Drs. Trojanowski and Lee Professors of Pathology
1. Name of the Equipment: Evolution P3 - Perkin Elmer
2. The Model Number - EP3B0031
3. The Year of Purchase – 2004

Dr. Dreyfuss, Professor of Biochemistry and Biophysics
1. Name of the Equipment - Beckman Coulter

2. The Model Number - Biomek FX
3. The Year of Purchase – 2002

Dr. Diamond, Professor of Engineering in the School of Arts and Sciences

1. Name of the Equipment - Perkin Elmer Janus Liquid Handler
2. The Model Number - AJL4001
3. The Year of Purchase – 2004

Dr. Ganguly, Associate Professor of Genetics

1. Name of the Equipment – Beckman Liquid Handling System
2. The Model Number - Biomek FX
3. The Year of Purchase – 2007

A6. Our reasons for choosing the Perkin-Elmer Janus Varispan + Modular Dispensing Tool (MDT) Workstation

While there are a handful of vendors that supply liquid handling workstations, such as Biomek, we chose the Janus from Perkin Elmer for the following reasons:

PerkinElmer's Janus fully automated liquid handling system is flexible covering a large breadth of Applications including: Plate Replication, Cherry Picking, Reformatting, Cell-based Assays, Elisa Assays, Reagent Addition, Serial Dilutions, DNA/RNA Purification, PCR Setup, Proteomics, Next Gen Sequencing and High Throughput Screening for both Small and Large Molecules. The modular design allows for easy integration of ancillary instruments for true walk-away automation. WinPREP, the Janus Operating Software was presented with an award by BioScience Technology for the "Easy to Use" and most user friendly software modules within the Liquid Handling and Automation World. This allows many scientists to operate the Janus with minimal training. PerkinElmer's "On the Fly" technology a patented technology held by PerkinElmer, truly separates the Janus from any other liquid handling instrumentation. This amazing technology enables the Janus to have the widest dynamic range on one system than any other liquid handler system on the market today. With MDT (Modular Dispense Technology) dispense heads can be automatically changes within a single protocol changing from nanoliter to microliters in seconds, from 96 to 384 to 1536 plate format as well as a wide range of tubes and vials. This can be accomplished within one protocol without user intervention. Most importantly, the "on the fly" MTD head is the major selling point as NO other manufacturer has this technology. Lastly, this is the instrument used by a number of academic and pharmaceutical screening cores. We discussed this with the NYU screening core, the Harvard screening core and the screening group at Merck. All of these screening Cores had positive feedback on this system.

Return to Section Headings Index
Return to Section Instructions – Justification of Need
Return to Section Guidelines – Justification of Need
Continue on to Justification of Need - Goldman Example

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.

(Figure 3)
(Figure 4)
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

1. Förster, Theodor (1948). "Zwischenmolekulare Energiewanderung und Fluoreszenz" [Intermolecular energy migration and fluorescence]. Annalen der Physik (in German) 437: 55–75.
2. L. Stryer, (1978) Fluorescence energy transfer as a spectroscopic ruler, Annu. Rev. Biochem. 47:819–846.
3. J. R. Lakowicz, (1983) Principles of Fluorescence Spectroscopy, Plenum Press.
4. David W. Piston, Gert-Jan Kremers (2007) Fluorescent protein FRET: the good, the bad and the ugly. Trends in Bio-chemical Sciences, 32:407–414.
5. Kim JY, Kim C, Lee NK. (2015) Real-time submillisecond single-molecule FRET dynamics of freely diffusing mole-cules with liposome tethering. Nat Commun. 6:6992.
6. Evangelos Sisamakis, Alessandro Valeri,Stanislav Kalinin, Paul J. Rothwell, and Claus A. M. Seidel. (2010) Accurate Single-Molecule FRET Studies Using Multiparameter Fluorescence Detection Methods in Enzymology, 475:455-514.
7. Stanislav Kalinin, Thomas Peulen, Simon Sindbert, Paul J Rothwell, Sylvia Berger, Tobias Restle, Roger S Goody, Holger Gohlke & Claus A M Seidel (2012) A toolkit and benchmark study for FRET-restrained high-precision struc-tural modeling. Nature Methods 9:1218-1225.

8. Suren Felekyan, Hugo Sanabria, Stanislav Kalinin, Ralf Kühnemuth, Claus A.M. Seidel (2013) Analyzing Förster Res-onance Energy Transfer with Fluctuation Algorithms. Methods in Enzymology, 519:39-85.
9. Felekyan, S., Kühnemuth, R., Kudryavtsev, V., Sandhagen, C., Becker, W. & Seidel, C. A. M. Full correlation from picoseconds to seconds by time-resolved and time-correlated single photon detection. Rev. Sci. Instrum. 76, 083104, (2005).
10. Jerker Widengren, Ülo Metz & Rudolf Rigler (1995) Fluorescence Correlation Spectroscopy of Triplet States in Solu-tion: A Theoretical and Experimental Study. J. Phys. Chem. 99:13368-13379.
11. Rüttinger S1, Macdonald R, Krämer B, Koberling F, Roos M, Hildt E. (2006) Accurate single-pair Förster resonant energy transfer through combination of pulsed interleaved excitation, time correlated single-photon counting, and fluorescence correlation spectroscopy. J Biomed Opt. 11:024012-024012-9.
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 elsewhere, 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 produce 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 re-quired 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 κ^2, 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 re-quest. 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 κ^2, 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 $\kappa 2$ value. For detection of dynamics of exchange 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 re-

vealed 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 performance, 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.

Return to Section Headings Index
Return to Section Instructions – Justification of Need
Return to Section Guidelines – Justification of Need
Continue on to Justification of Need - Sharp Example

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.

Return to Section Headings Index
Return to Section Instructions – Justification of Need
Return to Section Guidelines – Justification of Need
Continue on to Technical Expertise

- *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.

Return to Section Headings Index
Return to Section Instructions – Technical Expertise
Return to Section Guidelines – Technical Expertise
Continue on to Technical Expertise - Cherry Example

Cherry - Technical Expertise

As noted above, the Dr. Sara Cherry will serve as the Scientific Director of the Core. Dr. Cherry is an Associate Professor in the Department of Microbiology who is a leader in high-throughput screening. She trained with Dr. Peter Schultz as an undergraduate, Dr. David Baltimore as a graduate student and Dr. Norbert Perrimon as a postdoctoral fellow. She has >10yrs of experience performing HTS screens using small molecules and genetic perturbants. She has also performed both well-based (luminescence) and image-based screens. She has collaborated with a large number of Investigators to perform screens across diverse biologies. Within her own laboratory she has provided infrastructure, libraries and expertise to perform HTS screens for >10 investigators since her recruitment to Penn in 2006. Dr. Cherry is well-suited to direct this Core and oversee its operation.

The Facility will be operated by an experienced Technical Director and laboratory staff will be highly cross-trained technical experts in all areas of the proposed Core. The Director will manage a staff of additional key personnel and provide individualized consultation to Penn faculty on high-throughput screening projects. The Director will be responsible for the day-today operation of the Core, including all aspects of project management including, assay development, compound management, HTS, data analysis, and post-screen validation studies. All facility personnel will report directly to the Director. We anticipate hiring one PhD level staff scientist by no later than year-3 and will evaluate additional hires yearly with the help of the Advisory Committees.

An internal and external Advisory Committee will be assembled to develop strategic plans that meet the scientific needs of Penn faculty. The Internal Advisory Committee will be chaired by Dr. Morris Birnbaum, Assistant Dean for Core Facilities. The committee will conduct annual reviews of Core use, conduct user feedback surveys and track finances and management.

B1. Key personnel

Sara Cherry, PhD PI and Scientific Director
TBN, PhD Core Technical Director
TBN Staff Scientist

The development of a Screening Core at PENN fulfills a clear unmet need for such studies in this institution. The Janus Workstation will be a cornerstone of this Core performing all of the liquid handling needs of such a

Core. To exemplify these clear needs there are 4 major users who represent 4 different Departments within the School of Medicine. These users will account for 40% of the usage. Furthermore, there are 15 minor users who altogether represent 9 different Departments across the School who will account for 50% of the usage. The goal is to support this group of 19 NIH funded investigators (accounting for 90% of the usage) along with additional users, with preference to the major users.

B2. Major Users of this Equipment/Core

Last name, First name	Department	NIH Grant Number	Est. % of Use	Hours per week	Accessories Needed				
Major Users					MTD	Gripper	Varispa	Stacker	Enclosure
Berger, Shelley	CDB	5-R01-CA-078831-15	9%	3.60					
		5-U54-HD-068157-03							
		5-R01-GM-055360-15			yes	yes	yes	yes	yes
		5-R01-NS-078283-03							
		2-P01-AG-31862-06A1							
Bushman, Frederick	Microbiology	5-R01-AI-082020-04	17%	6.80					
		5-UH3-DK-083981-04							
		5-U19-AI-082628-05							
		5-P01-HL-059407-15							
		5-U01-HL-098957-05			yes	yes	yes	yes	yes
		5-U54-HL-117798-02							

B3. Minor Users of this Equipment/Core

Last name, First name	Department	NIH Grant Number	Est. % of Use	Hours per week	Accessories Needed				
Minor Users					MTD	Gripper	Varispa	Stacker	Enclosure
Alwine, James	Cancer Biology	5-R01-CA-157679-03	3%	1.20	yes	yes	yes	yes	yes
		5-R01-CA-157846-02							
Bates, Paul	Microbiology	5-R01-AI-081913-04	1%	0.40	yes	yes	yes	yes	yes

B4: Biosafety

Please note that none of the proposed projects require biosafety protocols for the use of this instrument. If there are requests to expand the coverage to include biosafety protocols our Scientific Advisory Committee will discuss this issue.

Return to Section Headings Index
Return to Section Instructions – Technical Expertise
Return to Section Guidelines – Technical Expertise
Continue on to Technical Expertise - Goldman Example

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, Chairman 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	Anisotropy	Quad detectors	Polarizers	Wavelengths (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	Anisotropy	Quad detectors	Polarizers	Wavelengths (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 interactions, 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.

References

1. Caporale A, Selis F, Sandomenico A, Jotti GS, Tonon G, Ruvo M. (2015) The LQSP tetrapeptide is a new highly effi-cient substrate of microbial transglutaminase for the site-specific derivatization of peptides and proteins. Biotechnol J.10, 154-161,
2. Cohen, JD, Zou, P, Ting, AY. (2012) Site-specific protein modification using lipoic acid ligase and bis-aryl hydrazine formation. ChemBioChem 13, 888-894.

3. Hegyi G, Michel H, Shabanowitz J, Hunt DF, Chatterjie N, Healy-Louie G, Elzinga M. (1992) Gln-41 is intermolecularly cross-linked to Lys-113 in F-actin by N-(4-azidobenzoyl)-putrescine. Protein Sci. 1, 132-144.
4. Mitra RD, Silva CM, Youvan DC. (1996) Fluorescence resonance energy transfer between blue-emitting and red-shifted excitation derivatives of the green fluorescent protein. Gene 173, 13-17,.
5. Milles S, Tyagi S, Banterle N, Koehler C, VanDelinder V, Plass T, Neal AP, Lemke EA (2012) Click strategies for sin-gle-molecule protein fluorescence. J. Am. Chem. Soc. 134, 5187-5195.
6. Nguyen T, Joshi NS, Francis MB (2006) An affinity-based method for the purification of fluorescently-labeled biomole-cules. Bioconjug Chem. 17, 869-872.
7. Pollok BA, Heim R (1999) Using GFP in FRET-based applications. Trends Cell Biol. 9, 57-60.
8. Benchaar SA, Xie Y, Phillips M, Loo RR, Galkin VE, Orlova A, Thevis M, Muhlrad A, Almo SC, Loo JA, Egelman EH, Reisler E. (2007) Mapping the interaction of cofilin with subdomain 2 on actin. Biochemistry 46, 225-233.
9. Taki M, Shiota M, Taira K (2004) Transglutaminase-mediated N- and C-terminal fluorescein labeling of a protein can support the native activity of the modified protein. Protein Eng Des Sel. 17, 119-126.
10. Wang L, Brock A, Herberich B, Schultz PG: (2001) Expanding the Genetic Code of Escherichia coli. Science 292,498-500.

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 $E_{FRET} = I_{ac} / (I_{ac} + \gamma I_{dc})$, where $I_{ac} = (I_a - b_a) - \beta(I_d - b_d)$ and $I_{dc} = (I_d - b_d) + \beta(I_d - b_d)$, b_a and b_d 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

FRET efficiency, E_{FRET}, is related to inter-probe distance, r, using the well-known Förster equations

$$E_{FRET} = \frac{1}{1+\left(r/R_0\right)^6} \quad \text{and} \quad R_0 = K \cdot \kappa^2 \cdot J$$

where K is a set of known physical constants, κ is an orientation factor that depends on the relative orientation and distance between the donor and acceptor, and J is an overlap integral that has a characteristic value for every donor-acceptor pair. The key to obtaining an accurate value of r from an E_{FRET} measurement is a dynamic model that provides an accurate value for κ.[1-4] Determining this value requires 3 types of information: (a) the relative orientations of donor and acceptor, (b) the relative positions of donor and acceptor to the protein attachment points, and (c) insight into the underlying protein dynamics involving those attachment points. If the relative orientations of donor and acceptor are random, or if their separation distance is large relative to R_0, then κ^2 is $2/3$. Under other conditions, its value can vary from 0 to 4 and must be known in order to reliably interpret E_{FRET} measurements in terms of r.

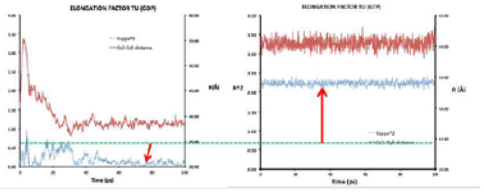

Fig. 7. Inter-fluorophore distance and κ^2 from molecular dynamics simulations of nucleotide-bound EF-Tu in which Cy3 is covalently attached to residue 47 and Cy5 to residue 314. Left: GDP. Right: GTP. The inter-fluorophore distance increases from an average of ~39 Å with GDP bound to ~64 Å with GTP bound, while the value of κ^2 increases from ~0.1 to ~2.2. The green line indicates $\kappa^2 = 2/3$.

It can be highly inaccurate to assume that κ^2 is $2/3$, or even that it does not change when an event such as ligand binding occurs. For example, our mo-

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.

References

1. Merchant,K.A., Best,R.B., Louis,J.M., Gopich,I.V. & Eaton,W.A. Characterizing the Unfolded States of Proteins Using Single-Molecule FRET Spectroscopy and Molecular Simulations. Proceedings of the National Academy of Sciences of the United States of America 104:1528-1533 (2007)
2. Best,R.B., Merchant,K.A., Gopich,I.V., Schuler,B., Bax,A. & Eaton,W.A. Effect of Flexibility and Cis Residues in Single-Molecule FRET Studies of Polyproline. Proceedings of the National Academy of Sciences of the United States of America 104:18964-18969 (2007)
3. Wozniak,A.K., Schroder,G.F., Grubmuller,H., Seidel,C.A.M. & Oesterhelt,F. Single-Molecule FRET Measures Bends and Kinks in DNA. Proceedings of the National Academy of Sciences of the United States of America 105:18337-18342 (2008)
4. Hoefling,M., Lima,N., Haenni,D., Seidel,C.A.M., Schuler,B. & Grubmuller,H. Structural Heterogeneity and Quantitative FRET Efficiency Distributions of Polyprolines Through a Hybrid Atomistic Simulation and Monte Carlo Approach. Plos One 6:(2011)
5. Mekler,V., Kortkhonjia,E., Mukhopadhyay,J., Knight,J., Revyakin,A., Kapanidis,A.N., Niu,W., Ebright,Y.W., Levy,R. & Ebright,R.H. Structural Organization of Bacterial RNA Polymerase Holoenzyme and the RNA Polymerase-Promoter Open Complex. Cell 108:599-614 (2002)
6. Margittai,M., Widengren,J., Schweinberger,E., Schroder,G.F., Felekyan,S., Haustein,E., Konig,M., Fasshauer,D., Grubmuller,H., Jahn,R. & Seidel,C.A.M. Single-Molecule Fluorescence Resonance Energy Transfer Reveals a Dynamic Equilibrium Between Closed and Open Conformations of Syntaxin 1. Proceedings of the National Academy of Sciences of the United States of America 100:15516-15521 (2003)
7. Andrecka,J., Lewis,R., Bruckner,F., Lehmann,E., Cramer,P. & Michaelis,J. Single-Molecule Tracking of MRNA Exiting From RNA Polymerase II. Proceedings of the National Academy of Sciences of the United States of America 105:135-140 (2008)
8. Muschielok,A., Andrecka,J., Jawhari,A., Bruckner,F., Cramer,P. & Michaelis,J. A Nano-Positioning System for Macromolecular Structural Analysis. Nature Methods 5:965-971 (2008)
9. Choi,U.B., Strop,P., Vrljic,M., Chu,S., Brunger,A.T. & Weninger,K.R. Single-Molecule FRET-Derived Model of the Synaptotagmin 1-SNARE Fusion Complex. Nature Structural & Molecular Biology 17:318-U84(2010)
10. Brunger,A.T., Strop,P., Vrljic,M., Chu,S. & Weninger,K.R. Three-Dimensional Molecular Modeling With Single Molecule FRET. Journal of Structural Biology 173:497-505 (2011)

11. Sabir,T., Schroder,G.F., Toulmin,A., McGlynn,P. & Magennis,S.W. Global Structure of Forked DNA in Solution Revealed by High-Resolution Single-Molecule FRET. Journal of the American Chemical Society 133:1188-1191
12. Mccann,J.J., Zheng,L.Q., Chiantia,S. & Bowen,M.E. Domain Orientation in the N-Terminal PDZ Tandem From PSD-95 Is Maintained in the Full-Length Protein. Structure 19:810-820 (2011)
13. Balci,H., Arslan,S., Myong,S., Lohman,T.M. & Ha,T. Single-Molecule Nanopositioning: Structural Transitions of a Helicase-DNA Complex During ATP Hydrolysis. Biophysical Journal 101:976-984 (2011)
14. Boura,E., Rozycki,B., Herrick,D.Z., Chung,H.S., Vecer,J., Eaton,W.A., Cafiso,D.S., Hummer,G. & Hurley,J.H. Solution Structure of the ESCRT-I Complex by Small-Angle X-Ray Scattering, EPR, and FRET Spectroscopy. Proceedings of the National Academy of Sciences of the United States of America 108:9437-9442 (2011)
15. Muschielok,A. & Michaelis,J. Application of the Nano-Positioning System to the Analysis of Fluorescence Resonance Energy Transfer Networks. Journal of Physical Chemistry B 115:11927-11937 (2011)
16. Olofsson,M., Kalinin,S., Zdunek,J., Oliveberg,M. & Johansson,L.B.A. Tryptophan-BODIPY: A Versatile Donor-Acceptor Pair for Probing Generic Changes of Intraprotein Distances. Physical Chemistry Chemical Physics 8:3130-3140 (2006)
17. Kalinin,S., Peulen,T., Sindbert,S., Rothwell,P.J., Berger,S., Restle,T., Goody,R.S., Gohlke,H. & Seidel,C.A.M. A Toolkit and Benchmark Study for FRET-Restrained High-Precision Structural Modeling. Nature Methods 9:1218-1225 (2012)

Return to Section Headings Index
Return to Section Instructions – Technical Expertise
Return to Section Guidelines – Technical Expertise
Continue on to Technical Expertise - Sharp Example

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.

Return to Section Headings Index
Return to Section Instructions – Technical Expertise
Return to Section Guidelines – Technical Expertise
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.

Return to Section Headings Index
Return to Section Instructions – Research Projects
Return to Section Guidelines – Research Projects
Continue on to Research Projects - Cherry Example

Cherry – Research Projects
Major User Research Project

C1. Berger, Shelley
PI Title: Professor of Cell & Developmental Biology
Grant No. and Title:
5-R01-CA-078831-15, PCAF/GCN5 Acetylation on P53 Transactivation
5-U54-HD-068157-03, Epigenetic Modification during normal and abnormal mammalian
5-R01-GM-055360-15, Chromatin Regulatory Mechanisms in Eukaryotic Gametogenesis
5-R01-NS-078283-03, Epigenetic Changes Associated with Neurodegenerative Diseases
2-P01-AG-31862-06A1, Epigenetics of Aging and Age-Associated Diseases

The Berger laboratory focuses on mechanisms that regulate gene expression with a special emphasis on how the DNA-packaging structure of chromatin is manipulated during genomic processes. Their findings inform the study of cancer and other diseases, and ultimately drug discovery. The primary projects in the lab include:

EPIGENETICS OF AGING AND AGE-ASSOCIATED DISEASES (2-P01-AG-31862-06A1)
Epigenetics is defined as heritable changes in genomic function and phenotype that do not involve alteration to DNA sequence. This higher level control of genome function is embodied in chromatin, a composite of nucleosomes (DNA and histones), as well as other non-histone proteins. Human disease is increasingly being linked to epigenetic and chromatin changes. A central hypothesis is that chromatin, as an inherently dynamic structure, is prone to age-associated degeneration, but that this degeneration is also countered by protective processes. These studies assess age-associated chromatin changes as they occur in the context of cell senescence, an irreversible proliferation arrest of damaged cells that contributes to tissue aging. Through a highly collaborative network they employ biochemistry, structural biology, cell biology, yeast genetics, and state-of-the-art epigenomic technologies in yeast and human cells to elucidate the role of epigenetics in aging and senescence. In particular, they define degenerative and protective changes to chromatin, and the molecular mechanisms underlying them.

Specific Benefits of the Proposal/Equipment: The Janus Workstation will greatly facilitate our ability to screen for chromatin modifiers that impact sensecence. The relevance of these studies for aging will be tested by reference to young and old human tissues and in mouse models, assessing phenotypes of aging. Moreover, based on our findings from the first cycle of funding, we have already initiated efforts to leverage our mechanistic insights into lead compounds for novel therapies to promote healthy aging. The Janus Workstation will facilitate our small molecule screening efforts. Our ultimate goal is to understand the balance of processes that culminate in age-associated chromatin dysfunction, so that we can devise strategies to manipulate the balance to promote healthy aging.

PCAF/GCN5 ACETYLATION ON P53 TRANSACTIVATION (5-R01-CA-078831-15)
The p53 tumor suppressor gene is frequently inactivated by mutations in human cancers. p53 is a sequence-specific transcription factor, whose activity is regulated by DNA damage, and activates expression of genes that induce cell cycle arrest or apoptosis. Regulation of p53 itself is complex and subject to DNA damage-regulated posttranslational modifications. These modifications include acetylation and phosphorylation. The lab has uncovered novel pathways for regulation of p53 via modifications, here they demonstrated that methylation and demethylation at lysine 370 (K370) are involved in regulating p53 in response to DNA damage. Preliminary data also show that a novel site, K373, is also methylated. Based on these observations, and methylation at additional p53 residues detected by others, they hypothesize that methylation of p53 serves to regulate p53 positively and negatively, and cross-talks with phosphorylation and acetylation. Lastly, they also uncovered an unanticipated novel pathway in the nucleus, where LKB1, the Peutz-Jeager kinase, and its downstream target, AMPK, function as transcriptional coactivators for p53. The kinases are directly recruited to p53-regulated

promoters and respond to multiple cellular stress pathways, including both DNA damage and metabolic stress. AMPK directly phosphorylates p53, and also phosphorylates a chromatin target, histone H2B. Based on these observations they propose that many enzymes carry out post-translational modifications of both factors, such as p53, and chromatin. For example, the serine/threonine kinase AMPK may function to target both p53 and histones in a coordinated fashion, and this may also be the case for lysine methyltransferases. In general, this coordination may lead to interrelated factor/histone modifications that reinforce one another in activating or repressing transcription. Ongoing studies will advance our understanding of mechanisms by which p53 activates gene expression in response to cellular stress, raising the likelihood for pharmacologic regulation of p53 function in human cancer (p53 methylation), diabetes and obesity (p53 phosphorylation) in the future.

Specific Benefits of the Proposal/Equipment:
The Janus Workstation will facilitate these studies as we can use the workstation to screen for small molecules and genes that impact the regulation of p53 by LKB1 and AMPK.

EPIGENETIC MODIFICATION DURING NORMAL AND ABNORMAL MAMMALIAN DEVELOPMENT (5-U54-HD-068157-03)

Dynamic epigenetic alteration is central to differentiation of mammalian sperm, however the nature of these changes largely remains unknown. The Berger lab proposes that sequentially altered patterns of histone posttranslational modifications underlies chromatin restructuring during spermatogenesis and in mature sperm. With collaborators they previously used sporulation in budding yeast S. cerevisiae, as a tractable model for gametogenesis, to uncover dynamic histone modifications, and then examined these in mouse spermatogenesis. These data indicate that mouse sperm development involves temporal sequences of histone modifications, including multiple novel modifications, which are analogous in timing to the yeast. This conservation of the pattern of histone modifications during gametogenesis from yeast to mammals, strongly indicates that epigenetic regulation is key to the normal process of chromatin restructuring during gametogenesis. They are now examining novel epigenetic regulatory pathways in normal and abnormal mammalian spermatogenesis, in the mouse model and in human samples. Their hypothesis is that chromatin modulation is a highly evolutionarily conserved process in gametogenesis, is a key regulatory feature of spermatogenesis, and is altered in abnormal sperm, including in human infertility. They will investigate histone modifications during normal and abnormal spermatogenesis in the mouse model and will examine sperm from human samples to determine whether modifications are altered.

Specific Benefits of the Proposal/Equipment:
The Janus Workstation will facilitate our ability to screen known chromatin modulating small molecules for affects on models of PAR metabolism.

CHROMATIN REGULATORY MECHANISMS IN EUKARYOTIC GAMETOGENESIS (5-R01-GM-055360-15)
Dynamic epigenetic alteration is central to differentiation of mammalian sperm, however the nature of these changes remains largely unknown. As noted above, the Berger lab is using the process of sporulation in the budding yeast S. cerevisiae, as a tractable model for mammalian spermatogenesis, to uncover dynamic chromatin and epigenetic regulation of transcription, meiosis and chromatin compaction. Previous observations indicate that there are dramatic temporal changes in chromatin during sporulation, including histone modifications and other alterations. Further results indicate that mouse sperm differentiation involves similar temporal sequences of histone modifications, which are analogous in timing to the yeast. This conservation of the pattern of histone modifications during gametogenesis from yeast to mammals, strongly indicates that epigenetic regulation is fundamental to the normal process of chromatin restructuring during gametogenesis. The working hypothesis is that chromatin modulation is a highly evolutionarily conserved process in gametogenesis, and thus is a key regulatory feature of both yeast sporulation and mammalian spermatogenesis.

Ongoing studies will address chromatin mechanisms during gametogenesis, through investigation of chromatin mechanisms that operate through histones H3 and H4, including novel post-translational modifications and other regulatory features, identified via mutational screening in the previous funding period; complete screening for histone substitution mutations in histone H2A and H2B that decrease or increase sporulation, and unravel their mechanisms through post-translational modifications, and other regulatory mechanisms, and linker histone Hho1-mediated mechanisms involved in meiotic gene transcriptional repression and post-meiotic chromatin compaction. As an important part of their studies they will determine whether these novel chromatin alterations are conserved during mouse spermatogenesis.

Specific Benefits of the Proposal/Equipment:
The use of the Janus workstation will facilitate mutational screening of histones and to identify compounds that disrupt posttranslational modifications. Overall, results from these studies will provide novel views of dynamic changes in chromatin structure and function.

EPIGENETIC CHANGES ASSOCIATED WITH NEURODEGENERATIVE DISEASES (5-R01-NS-078283-03)
Many fundamental cellular processes are affected by epigenetic modulation, and in recent years it has become evident that chromatin-based epigenetic mechanisms underlie important aspects of the aging process. However, despite the fact that age is a prominent risk factor in neurodegenerative disease (ND), there is remarkably little information on the role of epigenetic alterations in mechanisms of ND such as Alzheimer's disease (AD), Parkinson's dementia (PD), frontotemporal degeneration (FTLD) or amyotrophic lateral sclerosis (ALS). The Berger lab believes that a detailed biological, mechanistic and molecular understanding of the epigenetic factors that are altered in human ND holds promise for an improved understanding of disease pathogenesis and for the development of novel therapeutic interventions. Their goals in this area are to: (1) investigate whether major epigenetic modifications (histone post-translational modifications) change in the context of different NDs using an extensive bank of human samples, and (2) to use their model systems to discover new epigenetic modifications that underlie ND disease.

Specific Benefits of the Proposal/Equipment:
These studies would benefit greatly from the use of the Janus Workstation as we can screen chromatin modifiers for their impacts on ND of these model systems. In the broader scientific and medical communities, this effort will promote discoveries of epigenetic mechanisms of ND to provide the foundation for new insights and novel clinical approaches to treat ND.

See Bibliography for Relevant References.

Minor User Research Project

Alwine, James PhD
PI Title: Professor of Cancer Biology
Grant No. and Title:
R01 CA157679: Cytomegalovirus-mediated modification of host cell metabolism.
R01 CA157846-02: Assembly compartment formation and nuclear alterations mediated by HCMV.

Dr. Alwine’s research centers on DNA viruses and how they manipulate cellular systems to their advantage during infection and transformation. He has made numerous significant contributions beginning with his postdoctoral work where he showed that Simian virus 40 (SV40) large T antigen regulated the transcription of its own gene, the first demonstration of transcriptional autoregulation in a eukaryotic system. During this period he also developed the RNA transfer technique which he named the Northern Transfer. He has continued to

study SV40 and expanded to human cytomegalovirus (HCMV). He studied transcriptional activation by both viruses showing that both SV40 large T antigen and the HCMV major immediate early proteins interact with the basal transcription complex and facilitate its formation on promoters. He has extended his studies to the analysis of viral polyadenylation signals and how they functioned. His work defined control elements both upstream and downstream of the AAUAAA polyadenylation signal which affect polyadenylation efficiency in both viral and cellular mRNAs.

Project Descriptions:
R01 CA157679: Cytomegalovirus-mediated modification of host cell metabolism. The goals are to determine the mechanism by which HCMV and other viruses alter cellular metabolism.
R01 CA157846: Assembly compartment formation and nuclear alterations mediated by HCMV.The goals are to determine the alterations in cellular functions that lead to the formation of the HCMV cytoplasmic assembly compartment and alterations in nuclear structure.

Both projects involve the use of siRNA libraries directed against viral and cellular mRNAs to screen for viral and cellular proteins that participate in the metabolic (CA157679) or ultrastructural (CA157846) alterations in infected cells. The High-throughput Screening Core equipped with the PerkinElmer Janus 96/384 MDT Automated liquid pipetting workstation would greatly advance the ability to efficiently do these experiments.

Return to Section Headings Index
Return to Section Instructions – Research Projects
Return to Section Guidelines – Research Projects
Continue on to Research Projects - Goldman Example

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.

References

1. Ma,J.Q., Komatsu,H., Kim,Y.S., Liu,L., Hochstrasser,R.M. & Axelsen,P.H. Intrinsic Structural Heterogeneity and Long-Term Maturation of Amyloid Beta Peptide Fibrils. ACS Chem.Neurosci. 4:1236-1243 (2013)
2. Watts,J.C., Condello,C., Stõhr,J., Oehler,A., Lee,J., DeArmond,S.J., Lannfelt,L., Ingelsson,M., Giles,K. & Prusiner,S.B. Serial Propagation of Distinct Strains of Aβ Prions From Alzheimer's Disease Patients. Proc.Natl.Acad.Sci.USA 111:10323-10328 (2014)
3. Stõhr,J., Condello,C., Watts,J.C., Bloch,L., Oehler,A., Nick,M., DeArmond,S.J., Giles,K., DeGrado,W.F. & Prusiner,S.B. Distinct Synthetic Aβ Prion Strains Producing Different Amyloid Deposits in Bigenic Mice. Proc.Natl.Acad.Sci.USA 111:10329-10334 (2014)
4. Lu,J.X., Qiang,W., Yau,W.M., Schwieters,C.D., Meredith,S.C. & Tycko,R. Molecular Structure of Beta-Amyloid Fibrils in Alzheimer's Disease Brain Tissue. Cell 154:1257-1268 (2013)
5. Petkova,A.T., Leapman,R.D., Guo,Z., Yau,W.-M., Mattson,M.P. & Tycko,R. Self-Propagating, Molecular-Level Polymorphism in Alzheimer's B-Amyloid Fibrils. Science 307:262-265 (2005)
6. Kim,Y.S., Liu,L., Axelsen,P.H. & Hochstrasser,R.M. Two-Dimensional Infrared Spectra of Isotopically Diluted Amyloid Fibrils From Aβ40. Proc.Natl.Acad.Sci.USA 105:7720-7725 (2008)
7. Kim,Y.S., Liu,L., Axelsen,P.H. & Hochstrasser,R.M. 2D IR Provides Evidence for Mobile Water Molecules in B-Amyloid Fibrils. Proc.Natl.Acad.Sci.USA 17751-17756 (2009)
8. Komatsu,H., Feingold-Link,E., Sharp,K.A., Rastogi,T. & Axelsen,P.H. Intrinsic Linear Heterogeneity of Amyloid Beta Protein Fibrils Revealed by Higher Resolution Mass-Per-Length Determinations. J.Biol.Chem. 285:41843-41851(2010)

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 reconfigured 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 incorporation of unnatural amino acids (Po and Deutsch, unpub. data). Fluorescently-tagged residues are accommodated 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.

References

1. Lu,J., Hua,Z., Kobertz,W.R., & Deutsch,C. Nascent peptide side-chains induce rearrangements in distinct locations of the ribosomal tunnel. J. Mol. Biol. 411, 499 -510 (2011).
2. Lu,J. & Deutsch,C. Folding zones inside the ribosomal exit tunnel. Nat. Struct. Mol. Biol. 12, 1123-1129 (2005).
3. Lu,J. & Deutsch,C. Regional discrimination and propagation of local rearrangements along the ribosomal exit tunnel. J. Mol. Biol. 426, 4061-4073 (2014).
4. Tu,L., Khanna,P., & Deutsch,C. Transmembrane segments form tertiary hairpins in the folding vestibule of the ribosome. J. Mol. Biol. 426, 185-198 (2014).
5. Woolhead,C.A., McCormick,P.J., & Johnson,A.E. Nascent membrane and secretory proteins differ in FRET-detected folding far inside the ribosome and in their exposure to ribosomal proteins. Cell 116, 725-736 (2004).

Return to Section Headings Index
Return to Section Instructions – Research Projects
Return to Section Guidelines – Research Projects

Continue on to Research Projects - Sharp Example

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).**

Return to Section Headings Index
Return to Section Instructions – Research Projects
Return to Section Guidelines – Research Projects
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.

Return to Section Headings Index
Return to Section Instructions – Summary Table(s)
Return to Section Guidelines – Summary Table(s)
Continue on to Summary Table(s) - Cherry Example

Cherry – Summary Tables
Major Users

Last name, First name	Department	NIH Grant Number	Project Title	Project Start Date	Project End Date	Est. % of Use
Berger, Shelley	CDB	5-R01-CA-078831-15	PCAF/GCN5 ACETYLATION ON P53 TRANSACTIVATION	1-Apr-99	31-Mar-15	9%
		5-U54-HD-068157-03	PROJECT 3: EPIGENETIC MODIFICATION DURING NORMAL AND ABNORMAL MAMMALIAN	1-Apr-13	31-Mar-16	
		5-R01-GM-055360-15	CHROMATIN REGULATORY MECHANISMS IN EUKARYOTIC GAMETOGENESIS	1-May-98	31-Mar-16	
		5-R01-NS-078283-03	EPIGENETIC CHANGES ASSOCIATED WITH NEURODEGENERATIVE DISEASES	1-Mar-12	31-Jan-17	
		2-P01-AG-31862-06A1	EPIGENETICS OF AGING AND AGE-ASSOCIATED DISEASES	15-Mar-08	30-Jun-18	
Bushman, Frederick	Microbiology	5-R01-AI-082020-04	MASSIVELY PARALLEL ANALYSIS OF INTEGRATION IN THERAPEUTIC GENE TRANSFER	15-Jun-09	31-May-14	17%
		5-UH3-DK-083981-04	DIET, GENETIC FACTORS, AND THE GUT MICROBIOME IN CROHN'S DISEASE	1-Aug-12	31-Jul-14	
		5-U19-AI-082628-05	SEQUENCING AND VIRAL EVOLUTION CORE	1-Apr-13	31-Mar-14	
		5-P01-HL-059407-15	DNA VIRUS AS VECTORS FOR CARDIOVASCULAR DISEASES	1-Jul-13	30-Jun-14	
		5-U01-HL-098957-05	HUMAN RESPIRATORY TRACT MICROBIOME IN HEALTH, HIV INFECTION AND HIV LUNG DISEAS	25-Sep-09	31-Jul-14	
		5-U54-HL-117798-02	PERSONALIZATION OF THERAPEUTIC EFFICACY AND RISK	1-Jun-13	31-May-17	

Minor Users

Last name, First name	Department	NIH Grant Number	Project Title	Project Start Date	Project End Date	Est. % of Use
Alwine, James	Cancer Biology	5-R01-CA-157679-03	CYTOMEGALOVIRUS-MEDIATED MODIFICATION OF HOST CELL METABOLISM	1-Apr-11	31-Mar-16	3%
		5-R01-CA-157846-02	ASSEMBLY COMPARTMENT FORMATION AND NUCLEAR ALTERATIONS MEDIATED BY HCMV	1-Jun-12	31-Mar-17	
Bates, Paul	Microbiology	5-R01-AI-081913-04	INTERACTIONS OF EBOLA VIRUS GLYCOPROTEINS WITH HOST CELLS	1-May-10	30-Apr-15	1%
Cherry, Sara	Microbiology	5-R01-AI-095500-02	DISSECTING THE ALPHAVIRUS ENTRY RECEPTOR NRAMP	1-Jul-12	30-Jun-16	10%
		5-P30-DK-050306-17	CENTER FOR DIGESTIVE AND LIVER DISEASES	1-Jul-13	30-Jun-17	
		5-R21-AI-103441-02	DEFINING THE DETERMINANTS ON THE ALPHAVIRUS RECEPTOR NRAMP REQUIRED FOR VIRUS	1-Feb-13	31-Jan-15	
		5-R01-AI-074951-07	THE ROLE OF AUTOPHAGY IN INNATE ANTI-VIRAL IMMUNITY IN DROSOPHILA	1-Jul-07	13-Dec-17	
Dang, Chi Van	Medicine	5-R01-CA-057341-23	C MYC TARGETS IN THE PATHOGENESIS OF HUMAN CANCERS	1-Sep-92	31-Dec-14	1%

Return to Section Headings Index
Return to Section Instructions – Summary Table(s)
Return to Section Guidelines – Summary Table(s)
Continue on to Summary Table(s) - Goldman Example

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

[Return to Section Headings Index]
[Return to Section Instructions – Summary Table(s)]
[Return to Section Guidelines – Summary Table(s)]
[Continue on to Summary Table(s) - Sharp Example]

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	

Return to Section Headings Index
Return to Section Instructions – Summary Table(s)
Return to Section Guidelines – Summary Table(s)
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:
      1. A description of the core facility/entity that will oversee the instrument
      2. The location and space where the instrument will reside, including any drawings as needed with any necessary renovations
      3. Discussion of the administration of the instrument including the oversight committee, instrument access, scheduling, and dispute resolution
      4. Composition and role of technical advisory committee
      5. 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.

Return to Section Headings Index
Return to Section Instructions – Administration (Organizational/Management Plan)
Return to Section Guidelines – Administration (Organizational/Management Plan)
Continue on to Administration (Organizational/Management Plan) – Cherry Example

---

Cherry - Organizational/Management Plan: As Principal Investigator of this grant and Scientific Director of the Core, Dr. Cherry has responsibility for scientific and administrative oversight of all the instruments housed in the facility. In this capacity, Dr. Cherry reports directly to Dr. Morris Birnbaum, Assistant Dean of the Perelman School of Medicine Core Facilities. School Cores are required to complete an annual user survey, scientific review, and undergo rigorous financial oversight and annual reporting. Core reports are reviewed directly with the Executive Vice Dean/CSO. Financial management of all school cores is conducted centrally by the Dean's Office business administration unit. The business unit is supervised by Mr. Paul Umbriac. Mr. Unbriac has over 10 years of experience in managing university finance targeted to research and core facility operations. Mr. Umbriac reports to Ms. Rebecca Cooke, Vice Dean for Administration at the School of Medicine.

The Core **Scientific Advisory Committee:**

Name	Department	Status
Brinbaum, Morris	Medicine (Assistant Dean)	Chair
Ross, Susan	Microbiology (Dept Chair)	Member
Foskett, Kevin	Physiology (Dept Chair)	Member
Marmorstein, Ronen	Biochemistry	Member
Cherry, Sara	Microbiology	Core Scientific Director (ex officio)

On an operational level, the PI/Core Director, and Scientific Advisory Committee will meet yearly to discuss access usage policies and fees. For usage, we have already established an internet-based reservation system for our cores that will be applied here. This 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 Core Scientific Director, Dr. Cherry, ultimately controls access to the sign-up calendar, so only users who have received training from Core staff are able to reserve equipment. This is managed on a daily level by the Technical Director of the Core (PhD level position-TBD). The reservation software allows for straightforward statistical analysis of usage and also provides the basis for our billing strategy. Monthly use is tracked by the Technical Director, and users are provided with documentation for confirmation prior to debiting of accounts.

The estimated budget for establishing this core in FY15-17 is attached. The initial start-up costs include equipment (the Janus Workstation being essential) and library purchases as follows. With the exception of the Janus MDT Workstation, these costs will be covered outright by the School of Medicine (see Dr. Gaulton's letter).

Projected Budget for Purchases:

YEAR 1	
Item	**Cost**
Janus MDT spotter *(current S10 proposal)*	($424,000)
High-content screening reader	$250,000
Multi-label Plate Reader	$125,000
Dispensors	$30,000
Plate Washer	$25,000
Biosafety Cabinets	$25,000
CO2 Incubators	$20,000
TC microscope	$20,000
Freezers	$60,000
Centrifuge	$15,000
Small lab equipment	$15,000
Compound libraries	$50,000
siRNA libraries (human and mouse)	$200,000
miRNA libraries (human and mouse)	$70,000
Total	**$905,000**
YEAR 2	
Item	**Cost**
shRNA library (human and mouse TRC)	$130,000
cDNA prep (MGC collection)	$50,000
Total	**$180,000**
YEAR 3	
Item	**Cost**
Additional small molecule libraries	$50,000
Total	**$50,000**
TOTAL	$1,135,000

In addition to the Technical Director, additional staff will be added in year 3+ depending on Core usage. To the extent possible, usage fees will be set by the Core Director and Advisory Committee to offset ongoing costs for maintaining and operating the equipment, as well as a portion of the salary for the Core 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) <u>Screen optimization/Consulting:</u>

For initial, exploratory consultations (Up to 2 hours) no charges will accrue. 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 ~$65.00/hour. Staff and equipment will be available for training, assay development and optimization. This is priced hourly depending on equipment and labor needs. Once trained, many pieces of equipment can be used without oversight.

B) Pilot screens and small scale screens:
A pilot screen of all validated assays will be performed prior to starting full-deck screens to ensure assay performance is maintained during plate scaling. These screens will be performed by the Core @ $3,000.

C) Full-deck: Full deck represents screening of a complete library (e.g., siRNA, small molecule, custom array of shRNA/cDNA clones…). These screens will be performed by the Core. These rates forecast costs associated with facility staff time, equipment usage, reagents (i.e., compound, siRNA), data management, analysis, and review. These costs do not include assay specific reagents (e.g. transfection reagent, detection reagents, etc.), which will be provided by the Investigator. These screens should be ~$12,000.

D) Post-screen experiments and Hit-picking: These are costs associated with cherry- picking active compounds/siRNA from screening decks to retest/confirm activity in an independent experiment. Actives can be retested at a single concentration of as a concentration series. These costs are experiment specific and influenced by the scope of the experiment: $500-$1,000/experiment.

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 the cost of consumable supplies. The anticipated Core operation costs and user fees (from faculty surveys) are listed below. We appreciate that even after Year-3 it may not be possible to fully recover the costs of Core operation, and the School of Medicine has committed through its central core support committee to underwrite such expenses (see Dr. Gaulton'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 enhanced revenues will allow for additional new initiatives and upgrades.

Costs for the phases of a screening project can be broken into several categories:

E) Three year Estimate of Facility Operational costs

	year 1	year 2	year 3
Salary and FB	$100,000	$100,000	$165,000
Data Storage (~3,000/TB) and IT	$10,000	$15,000	$20,000
Equipment service	$10,000	$20,000	$30,000
Supplies	$20,000	$40,000	$50,000
Software and license fees	$10,000	$10,000	$10,000
Library upgrades, replacements, etc			$30,000
Estimated User Fees	($40,000)	($120,000)	($240,000)
Total Costs	**$110,000**	**$65,000**	**$65,000**

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 Core may build full competency and establish its user base.

Once steady state is achieved, we expect major users to account for approximately 80% of total usage for the requested Perkin-Elmer Janus Varispan + Modular Dispensing Tool (MDT) Workstation, and they will always have priority in reserving microscope time. Any disputes in Core usage will be resolved by the Advisory Committee. As a key component of our Core this instrument will also be available to researchers outside the major user group, who will be charged at the rates described above. As indicated previously in the proposal, 19 users have already expressed a strong interest in utilizing the Screening Core services. Lastly, as the

publications listed by our past and present users indicate, we have demonstrated the strong need to establish this facility and the central importance of this equipment request.

Return to Section Headings Index
Return to Section Instructions – Administration (Organizational/Management Plan)
Return to Section Guidelines – Administration (Organizational/Management Plan)
Continue on to Administration (Organizational/Management Plan) – Goldman Example

---

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 us-age 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.

Return to Section Headings Index
Return to Section Instructions – Administration (Organizational/Management Plan)
Return to Section Guidelines – Administration (Organizational/Management Plan)
Continue on to Administration (Organizational/Management Plan) – Sharp Example

---

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.

Return to Section Headings Index
Return to Section Instructions – Administration (Organizational/Management Plan)
Return to Section Guidelines – Administration (Organizational/Management Plan)
Continue on to Institutional Commitments

- *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.

Return to Section Headings Index
Return to Section Instructions – Institutional Commitments
Return to Section Guidelines – Institutional Commitments
Continue on to Institutional Commitments – Cherry Example

Cherry – Institutional Commitments

F. Institutional Commitments

We have included strong letters of support from:

1) Chair of Microbiology, Dr. Susan Ross

2) Executive Vice Dean and Chief Scientific Officer of the School of Medicine, Dr. Glen Gaulton

Return to Section Headings Index
Return to Section Instructions – Institutional Commitments
Return to Section Guidelines – Institutional Commitments
Continue on to Institutional Commitments – Goldman Example

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.

Return to Section Headings Index
Return to Section Instructions – Institutional Commitments
Return to Section Guidelines – Institutional Commitments
Continue on to Institutional Commitments – Sharp Example

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.

Return to Section Headings Index
Return to Section Instructions – Institutional Commitments
Return to Section Guidelines – Institutional Commitments
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.

Return to Section Headings Index
Return to Section Instructions – Overall Benefit
Return to Section Guidelines – Overall Benefit
Continue on to Overall Benefit – Cherry Example

Cherry – Overall Benefit

G. Overall Benefit

The aim of this proposal is to purchase a state-of-the-art automated liquid handling workstation that will be the cornerstone of our new Screening Core.

As Penn investigators are leaders in biomedical research, a centralized HTS facility is needed to support broadly the growing demands for chemical and genetic screens by Penn faculty. No unified shared resource exists at Penn that can support these needs. The centralization of these services in a core setting takes advantage of highly specialized laboratory infrastructure, including laboratory robotics, screening libraries, computational infrastructure for analysis and interpretation of biological and chemistry data sets, and highly trained key personnel.

Therefore, there will be several major benefits for the acquisition of this Janus Workstation at the Core:

1) The Core will provide access to robotic liquid handling and provide expert training on its use.

2) The Core will support the research activity of 4 Major users and 19 Minor users who are NIH-funded ($21M) across the School of Medicine.

3) The Core will generate revenue to pay for the service contracts and salaries of the Core. This will maintain the mission of the Core long term.

Return to Section Headings Index
Return to Section Instructions – Overall Benefit
Return to Section Guidelines – Overall Benefit
Continue on to Overall Benefit – Goldman Example

Goldman – Overall Benefit

G. Overall Benefit

The aim of this proposal is to purchase a state-of-the-art novel microscopy workstation for advanced struc-tural 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.

Return to Section Headings Index
Return to Section Instructions – Overall Benefit
Return to Section Guidelines – Overall Benefit
Continue on to Overall Benefit – Sharp Example

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.

Return to Section Headings Index
Return to Section Instructions – Overall Benefit
Return to Section Guidelines – Overall Benefit
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) and *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.

Return to Section Headings Index
Return to Section Instructions – Letters of Support and Other Attachments