The Rockefeller University Resource Centers
Descriptions of facilities/resources (for use in grant applications)

Please feel free to contact the director(s) of the resource center(s) if you need more specific information about how your work can be supported by the resource center, including information about the appropriateness and/or availability of collaborative endeavors and/or letters of support.

Bio-Imaging Resource Center

The Bio-Imaging Resource Center provides researchers with training, advice and access to instrumentation and image analysis software for state-of-the-art optical imaging using widefield, confocal, multiphoton, and super-resolution fluorescence. Consultation on sample preparation and immunolabeling procedures is also provided.. Researchers are trained to use the microscopes and image acquisition software themselves, with staff assistance when necessary to ensure collection of high quality images. More complex work can also be performed on a collaborative basis with the staff of the center.

The center currently houses several widefield fluorescence/transmitted light microscopes (Zeiss and Olympus), two DeltaVision Image Restoration microscopes (API), four laser scanning confocal microscopes (one Zeiss LSM 780, one Leica SP5 and two Zeiss LSM 510 systems) , a Yokagawa spinning disk confocal microscope (Zeiss/Spectral Applied), an FV1000MPE Twin upright multiphoton system (Olympus), an LSM 510 NLO inverted multiphoton system (Zeiss), a multi-line STORM/TIRF system (Nikon) fitted with widefield-FLIM and TIRF-FLIM capabilities (Lambert Instruments), an LCV110 "VivaView" Incubator Microscope (Olympus), a laser microdissection system (MMI), and an OMX 3D-SIM super-resolution microscope (API). Most of the systems are fitted with environmental chambers for live cell imaging.

The center has four full-time research support staff including the senior director, Alison North, Ph.D., who has led the center since 2000. Dr. North received her Ph.D. in cell biology from Oxford University and has over 20 years of experience in light and electron microscopy. The center is staffed during business hours and under an open-access model, trained investigators can use the facility 24/7. In addition, many of the systems can be operated remotely via the center's on-line facility management software.

Priority access is given to researchers from The Rockefeller University, which provides significant financial subsidy for the center's operations, but the center is also open to researchers from other not for profit institutions. The BIRC is located in approximately 2900 square feet of recently renovated space in the Bronk Building on The Rockefeller University campus.

Flow Cytometry Resource Center

The Flow Cytometry Resource Center (FCRC) provides University investigators with equipment and support for cell sorting (separation), acquisition, and analysis of flow cytometric data. The FCRC has a wide variety of state-of-art multi-laser/multi-color flow cytometry sorters and analyzers, as well as an imaging flow cytometer. The FCRC four full time staff members maintain the instruments, assist with experimental design and troubleshooting, advise on sample preparation, consult on data analysis and provide individualized training.

Cell sorting is performed by FCRC staff on three BD FACSAria cell sorters equipped with up to six-lasers (488, 561, 640, 355, 405, and 445 nm excitation wavelengths) and 18 fluorescence channel detection. All the cell sorters are able to perform high-speed (up to 20,000 events/s), high-purity (up to 99.9%) sterile sorts into different types of devices (tubes, plates, slides, or custom-made devices). The ImageStream-X combines the strength of flow cytometry and fluorescence microscopy in a single platform. It allows for high content assays on rare cells and quantification of biological phenomena with incredible accuracy. The ImageStream-X is able to simultaneously record up to 10-color images with the four-laser excitation (488, 561, 658, and 405 nm wavelengths). Data acquisition on ImageStream-X is performed by FCRC staff.
The FCRC is equipped with two advanced benchtop analyzers (BD LSRIIs) equipped with up to five-lasers (488, 561, 640, 355, 405 nm, and 445 excitation wavelengths) and 16 fluorescence channels. The FCRC also has two basic analyzers, a BD FACSCaliburs with two-lasers/four-channels and a BD Accuri C6 flow cytometer. After completion of appropriate equipment training, researchers have 24/7 access to and can operate the analyzers themselves.

The FCRC, located in approximately 1700 square feet of recently renovated space in the Bronk Building on The Rockefeller University campus, is directed by Svetlana Mazel, who received her Ph.D. in immunology from the Gabrichevsky Institute for Epidemiology and Microbiology in Moscow, has over 17 years of experience in flow cytometry, and has been directing the FCRC since 2001.

Genomics Resource Center
The Genomics Resource Center offers comprehensive services and state-of-the-art instruments to support genomics research. The approximately 3000 sq. ft. center houses all three major microarray platforms (Affymetrix, Agilent, and Illumina). two Illumina HiSeq 2000 next-generation DNA sequencers, two realtime PCR systems (ABI 7900 HT and Roche LightCycler), and several accessory instruments for sample quantity and quality validation. The center offers full support for whole genome gene expression and SNP genotyping analysis on microarrays, including sample preparation, array hybridization and processing, and data analysis.

For the next-generation DNA sequencing service on Illumina HiSeq 2000, users can either provide their own libraries or use the center's library preparation service for genomic DNA-seq and RNA-seq. The center performs initial sequencing data analysis for all users, and can assist with downstream analysis.

The center is staffed by six full time personnel and is directed by Connie Zhao, Ph.D. Dr. Zhao received her PhD in molecular genetics from Albert Einstein College of Medicine and did postdoctoral studies with Jeff Friedman at The Rockefeller University. She has led the center since 2003. In her role as the director of the GRC, Dr. Zhao has been very successful in implementing new technologies, adding SNP genotyping, micro RNA profiling, and next-generation DNA sequencing platforms. She is an active participant in initiatives related to genomics through AMDeC, the New York Genome Center, and the American Society of Human Genetics.

High-Throughput Screening Resource Center
(http://www.rockefeller.edu/htsrc)

The High-Throughput Screening Resource Center (HTSRC) supports scientists in the screening and identification of compounds and genetic modulators of in vitro assays which utilize optical and other bio-analytical technologies. The center has a collection of 169,000 compounds, automated liquid transfer devices, compound databases, and supports a broad diversity of assay development techniques, typically found in early drug discovery programs. The High-Throughput Screening Resource Center also oversees the management of, training for and scheduling for optical spectroscopic and calorimetric equipment for use in studies of the structure, function and interactions of biological and organic molecules.

The center is staffed full-time under an open-access model, whereby, once trained, investigators can use the facility 24/7. The staff includes a director with over 20 years of screening and drug discovery experience in the pharmaceutical industry, who is readily available for scientific and technical consultations, and three research associates, who support the automation, data processing, liquid handling and instrument QC and maintenance. The Center has the capacity to support approximately 15 full high throughput screening (HTS) projects per year, in addition to various pre-HTS and post-HTS activities.

The center is directed by J. Fraser Glickman who has a Ph.D. in biochemistry from UC Santa Barbara and over two decades of experience in drug discovery and HTS including a decade working in the pharmaceutical industry. Dr. Glickman has been directing the HTSRC since 2008.

The center occupies 1500 sq. ft. of lab space located on the Rockefeller campus on the Upper East Side of Manhattan in proximity to Weill-Cornell Medical College, Memorial-Sloan Kettering Cancer Center and the NYU Langone Medical Center. The lab space is equipped with assay development workbenches and data analysis workstations, analytical instruments, electronic automated pipetters and houses a full cell culture facility. Cell dispensing can be done under full laminar flow biosafety cabinets, and the center is certified for work at BSL2.

Compound Library

The Rockefeller University Compound Collection consists of 169,000 compounds from a variety of commercial vendors and academic sources (for example ChemDiv, ChemBridge, Cerep, Prestwick, Sigma, Analyticon, LifeChemicals, Enamine, Biofocus, AMRI and the Laboratories of Dr. Derek Tan). The purchasing strategy strives to include all of the well-known forms of compound synthesis and acquisition, including solid-phase pool-and-split, parallel synthesis, individual synthesis, semi-synthetic approaches and natural product isolation. Compounds are chosen using Lipinski “rule-of-five,” removing reactive substructures, dyes and known frequent hitter scaffolds. Diversity algorithms (such as connectivity fingerprints, Tanimoto based scoring) are used to increase the compound diversity, while maintaining the potential for structure-activity-relationships. Most of the compounds can be readily re-ordered in larger batches for hit follow-up and secondary assays. Analytical data is required of all purchased compounds such that a minimum of 95% purity is met.

The library is stored as 10 to 15 copies of 5 millimolar solutions in DMSO, heat-sealed and barcoded 384-well small-volume polypropylene microtiter plates. At least 8 archival copies are stored at -30^{o}C in REVCO freezers with emergency power and the two working copies are stored at -20^{o}C in a HighRes Biosolutions NanoCell system. The NanoCell system is comprised of a Liconic dry-storage random access deep freezer, a 6-axis robotic arm, an automated heat-

sealer and de-sealer, a NanoServe plate carousel and barcode reader. Cellario scheduling software allows the system to deliver hundreds of library plates in short order. A "micro-dock" system allows for flexible integration of the screening instruments. The "working" copies of the library are used for a maximum of 10 freeze-thaw cycles. High-throughput cherry picking and serial dilution is accomplished with a Perkin-Elmer Janus automated pipette system capable of accurately selecting and arraying 1000 picks of 0.5 microliter of compound solution per day. Sample integrity of the library is periodically monitored by HPLC-MS of random samples.

RNAi Libraries

The Rockefeller University HTSRC and the RNAi Core Facility at the NYU Langone Medical Center have agreed to share resources. The understanding between the two Centers allows HTSRC members access through the NYU Core, to the following libraries

- Human annotated genome (siRNA library from Ambion)
- Human pre-miR and anti-miR miRNA collection (from Ambion)
- Mouse druggable genome (siRNA library from Ambion)
- *C. elegans* whole genome (2 bacterial feeding libraries from Vidal and Ahringer collections)

Instruments

The Center is configured for processing 96, 384 and 1536-well microtiter plates through the use of automated changeable tip dispensers, non-contact dispensers, and syringe dispensers. For assay technologies, the HTSRC has the capability to support cellular and biochemical assays using absorbance, fluorescent kinetics, fluorescence anisotropy, time-resolved fluorescence, time-resolved fluorescence resonance energy transfer, AlphaScreen, bioluminescence, for example luciferase and green fluorescent protein, scintillation proximity and cellular fluorescence imaging. Assay targets can include ion channels, receptors, enzymes, protein interactions, signaling pathways and cellular processes.

Plate Readers

Perkin-Elmer Envision

fluorescence polarization, time gated fluorescence, prompt fluorescence luminescence, alphascreen

384 wells / 2 minutes

Hammmatsu FDSS 6000

Fluorescence Kinetics, calcium flux assays

384 wells in 2-4 minutes

Flexstation II Dual Monochromator

Fluorescence Kinetics, calcium flux assays

96 wells in 2-4 minutes

Perkin-ElmerTriLux (Scintillation and Luminescence)

384 wells in 30-40 minutes scintillation

384 wells in 5 minutes luminescence

Molecular Devices ImageXpress Micro

Fluorescence Microscopic Imaging

384 wells in 15-40 minutes fed with a Thermo CRS robot arm driven by POLARA scheduling software. MetaXpress software can analyze and score imaging for morphological and

subcellular events, such as translocation, spot formation and process outgrowth. The detection system is based on a fluorescence microscope with automatic laser focusing.

Molecular Devices Analyst GT

fluorescence polarization, time gated fluorescence, prompt fluorescence, luminescence
384 wells in 2 minutes

Molecular Devices Image Express Velo Laser Scanning Cytometer

This two color (488nm and 534nm) laser-based scanning imager can image 384 wells in 5-10 minutes. The system is equipped with a Twister II robot arm such that 40 plates can be processed unattended. The software can score fluorescent intensity per cell , number of cells, surface area of cells, ellipticity of cells in up two three individual emission bands.

Biotek synergy H4 with Biostack Plate Feeder

A multi-purpose high-speed microplate reader with dual monochromators which can allow the user to dial in the particular wavelengths of interest. Can read alphaLisa, TR-FRET, assays. Equipped with in-line injection port which allows for rapid kinetic analyses.

Analytical Instruments

The following instruments and associated techniques are available: a Fourier transform infrared spectrometer (FTIR), a circular dichroism spectrometer (CD), a surface plasmon resonance (Biacore 3000) detector (SPR), a scanning spectrofluorometer and an isothermal calorimeter (GE AutoITC 200).

Fluidics and Automated Pipetters

Perkin-Elmer Janus Automated Workstation

Disposable tips or nano head syringes are used for compound dispensing in the 50 nL-50 uL range, 96, 384, or 1536-well formats

Perkin-Elmer Janus Mini

Disposable tips or nano head syringes
50 nL-20 uL, 96 or 384-well formats

Thermo MultiDrop Combi **with 50 plate feeders**

This is a Non-contact, peristaltic plate filler capable of dispensing 1uL-200uL of solution or suspension in 96, 384, or 1536 well formats with high precision (CV <5%) . 50 plates can be loaded onto the stacker, and fed with a flask of single cell suspension, in 50 minutes. The peristaltic tubing system used to control the volume dispensed has no effect on cell viability in microtiter plates. The Multi-drop combi is housed in a Biosero BigNeat Laminar flow HEPA-filtered biosafety cabinet such that sterility and safety is maintained. The replaceable tubing cassette is completely autoclavable.

Union Biometrica COPAS™ Select

For sorting large particles into microtiter plates

Biotek EL406 Microtiter Plate Washer with BioStack automated plate feeder

Washes a 96/384 well plate in 30 seconds or less, with precise control of flow rates and tip distances.

Informatics

All screening data can be normalized, stored, processed and queried using the *Collaborative Drug Discovery* Database (CDD, Burlingame California). The database can be accessed by all users and data remains separated by project. Using this database, calculated properties, frequent hitters and heatmap displays are easily viewed and compound profiles can be determined using cross screen analysis. Statistical values such as Z and Z-prime can readily be

calculated. Studies of the Structure-Activity Relationships, similarity and sub-structure searches and Bayesian predictions can be accomplished using *Accelrys Pipeline Pilot* software. Additionally, a subscription to the *ChemNavigator* Database is maintained. This database allows for searching and procurement of over 50 million commercially available compounds for follow-up chemistry.

Monoclonal Antibody Resource Center

The Memorial Sloan Kettering Cancer Center-Rockefeller University Monoclonal Antibody Resource Center generates custom monoclonal antibodies (MAbs), produces large scale quantities of monoclonal antibodies and provides a number of related and ancillary services on a fee for service basis. Grounded in a thorough understanding of MAb technology, the Center's staff provide comprehensive oversight and are available for consultation throughout the custom MAb generation process. Custom MAb services include, but are not limited to, animal immunizations, B cell immortalization, maintenance of the hybridomas during the screening process, ELISA screening and establishment of stable antibody producing cell lines. Driven by individual laboratory needs, the Resource Center produces 10 - 4,000 mg quantities of over 400 various monoclonal antibodies as well as providing antibody conjugation and fragmentation services. To support the maintenance of clean cell cultures, the Center offers a weekly mycoplasma testing service, complemented by consultation on how best to address this common contaminant. The Center is headed by Frances Weis-Garcia, Ph.D., who has over 20 years' experience in immunology and monoclonal antibody biology, supported by four additional full time staff members, occupies about 1,250 square feet of space, and has facilities located on both the MSKCC and Rockefeller University campuses to ensure easy access to all researchers. Based on capacity and work load, researchers from other not-for-profit research institutions can access the Resources Center services as long as Rockefeller and MSKCC research laboratories have first priority. The Monoclonal Antibody Resource Center currently supports over 100 research groups at Rockefeller and MSKCC spanning various biological fields.

Proteomics Resource Center (PRC)

The Proteomics Resource Center operates an Orbitrap mass spectrometer equipped with nanoflow HPLC, in addition to several other mass spectrometers, including a triple quadruple for validation experiments. A Q-Exactive mass spectrometer has been purchased and is expected to be fully commissioned in early 2012.

The Center offers quantitative proteomics services based on metabolic labelling (SILAC: *stable isotope labeling with amino acids in cell culture*), chemical labelling (dimethyl), or absolute quantitation (multiple reaction monitoring). Relative quantitation based on the tandem-mass tag technology is being implemented.

In addition to analysis by mass spectrometry, the Center offers production of custom peptides and peptide libraries and different pre-mass spectrometry fractionation techniques, which include off-line separation of peptides and proteins by liquid chromatography and off-gel

electrophoresis, as well as user access to a MALDI-TOF mass spectrometer, a Typhoon variable mode imager, HPLC and FPLC.

The Proteomics Resource Center is directed by Henrik Molina, Ph.D. who oversees five scientist staff members. Dr. Molina has more than a decade of experience in most aspects of mass spectrometry based proteomics. Dr. Molina's experience includes five years in the biotech industry, six years at The Johns Hopkins University and three years as the Director of the Proteomics Unit at the Center for Genomic Regulation in Barcelona, Spain, prior to his arrival at the University in 2011.

The Center occupies over 3000 sq. ft. of lab space located on the Rockefeller campus on the Upper East Side of Manhattan in proximity to Weill-Cornell Medical College and Memorial-Sloan Kettering Cancer Center.

The Spectroscopy Resource Center (SRC)

The Rockefeller University maintains on-campus facilities for nuclear magnetic resonance spectroscopy with priority access given to University researchers. The Center has two nuclear magnetic resonance spectrometers (one 400 MHz with a BBFO probe and one 600 MHz with a TCI cryoprobe) for use in studies of the structure, function and dynamics of macromolecules, as well as spectroscopic fingerprint of peptides and small organic molecules. The Center offers extensive training in basic and advanced NMR experiments and also coordinates access to high field spectrometers (700, 800, and 900 MHz) at the New York Structural Biology Center through the University's partnership in that Center. SRC staff provide general background training in applications of NMR spectroscopy to structural biology and chemistry and hands on training on the spectrometers. Trained users have 24/7 access to these instruments. Occasional users of the facilities may have spectra produced for them.

The Structural Biology Resource Center (SBRC)

The SBRC houses instruments and provides staff with expertise needed to pursue three-dimensional structure determination using X-ray crystallography. The center staff provides training on instrument use and advice on experimental design and implementation. The facility instrumentation includes two liquid handlers (the Phoenix by Art Robbins and the Formulator by Formulatrix) that allow for increased speed and efficiency of crystallization experiments as well as the ability to proceed with projects using much smaller quantities of protein and a UV fluorescence crystal-evaluation station (the UVEX by JANSi) with an in-house X-ray generator (MM007/RaxisIV++ by Rigaku) for crystal testing and data collection. The center, generously subsidized by the University, offers consultations at all stages of structure determination as well as coordination with and accompanying support at the national synchrotron light sources. Under special agreements made by the University, researchers have direct access to X29 of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory and 24ID of the Advanced Photon Source at Argonne National Laboratory. The SBRC is managed by Deena A. Oren, a protein crystallographer, who obtained her master's degree at the Hebrew University in Jerusalem and her Ph.D. at Rutgers University under the guidance of

Dr. Eddy Arnold. She had been responsible for X-ray facilities at the Hebrew University after her post-doctoral work and at Rutgers University prior to joining The Rockefeller University.