5/20/2016

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, light-sheet and super-resolution microscopy. 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/GE Healthcare), three laser scanning confocal microscopes (a Zeiss LSM 780, a Leica SP8 and a Zeiss LSM 880 NLO system), a Yokagawa spinning disk confocal microscope (Zeiss/Spectral Applied), an FV1000MPE Twin upright multiphoton system (Olympus), a multi-line STORM/TIRF system (Nikon) fitted with widefield-FLIM and TIRF-FLIM capabilities (Lambert Instruments), an LCV110 "VivaView" Incubator Microscope (Olympus), a Yokawawa CV1000 “CellVoyager” environmental spinning disk system (Olympus), an Ultramicroscope light sheet system (LaVision BioTec), a laser microdissection system (MMI), and an OMX 3D-SIM super-resolution microscope (API/GE Healthcare). 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 external institutions. The BIRC is located in approximately 2900 square feet of recently renovated space in the Bronk Building on The Rockefeller University campus.

Cryo-Electron Microscopy Resource Center

The Evelyn Gruss Lipper Cryo-Electron Microscopy Resource Center (CEMRC) provides its users with a world class environment to make advances in structural biology. The center is equipped with the two highest end dedicated cryo-electron microscopes commercially available. The microscopy suite was meticulously and specially designed to allow these instruments to perform beyond their specified resolutions. This center provides users with the world’s most stable dedicated cryo-electron microscopes optimized for high resolution single particle analysis of proteins and protein complexes as well as high resolution cellular tomography. Users will work alongside CEMRC staff until they have mastered electron microscope operation at which point they will be able to work autonomously.

The center is home to the FEI Talos Arctica, a 200kV transmission electron microscope (TEM), and the FEI Titan Krios, a 300kV TEM. Both systems are equipped with an autoloader, allowing storage and

automated transfer for up to 12 frozen hydrated samples at once. These systems can be run completely autonomously as well as remotely, providing the ability for 24/7 operation. Both systems are equipped with high brightness field emission guns (FEGs). These FEGs produce coherent electrons, aiding in boosting contrast for data acquisition. The Krios and Arctica are fitted with Gatan K2 Summit direct electron detectors. These cutting edge complementary metal oxide semiconductor (CMOS) based detectors make it possible to visualize the most difficult low contrast particles through their excellent detective quantum efficiency (DQE). The CMOS chips on Rockefeller's K2 cameras are Gatan's latest generation, allowing for data collection under the best DQE curves available with current technology. These tools are tailored for high throughput data collection of 2D and 3D images.

The CEMRC suite is located in the basement level of Collaborative Research Center. Priority access to the CEMRC is given to Rockefeller scientists and the University generously subsidizes the cost of operation. The center is directed by Mark Ebrahim. Mark has over 10 years of combined multi-disciplinary research and industry experience in the fields of materials science, physical biology, and electron optics. Mark received this master's degree in physics concentrating in optics and ultrafast laser spectroscopy at CUNY Hunter College.

Electron Microscopy Resource Center

The Electron Microscopy Resource Center offers state-of-the-art instrument and distinguished expertise, thereby it can provide educational and technical supports for a broad range of cutting edge ultrastructural studies. Brainstorming, assay planning and running experiments, as well as training on instruments/methodology and analyses are available. The studies can be purely structural analysis or integration of structural and functional correlative studies with other disciplines, such as light microscopy, genetics, proteomics, or structural biology. Users can request training for operation of a device or application of methodology, alternatively can request experiments to be ran by staff.

The EMRC has three transmission electron microscopes (TEM): JEOL 100-CX, JEOL JEM1400 Plus, FEI TECNAI Bio-twin G2, and two scanning electron microscopes (SEM): Zeiss Merlin and Zeiss Leo1550. The JEM1400 Plus and TECNAI G2 have automated tomography and montage capabilities for both ambient and cryo conditions. In addition, the JEM1400 plus is equipped for Scanning Transmission Electron Microscopy (STEM) and Energy-Dispersive X-ray Spectroscopy (EDS). Zeiss Merlin is a high end SEM that is equipped for 3D EM, which can be done by serial Block Face imaging with the automated slicing device (Gatan 3View-2XP) or by array tomography with the automated STEM imaging system (Fibics Atlas). The EMRC also offers collection of accessory devices; including Edwards Vacuum Evaporator 306A, Leica Spatter Coater ACE600, Leica High Pressure Freezer EMPACT2/Freeze Substitution unit AFS, microwave system (PELCO BioWave) and ambient or cryo-Ultramicrotomes.

The EMRC staff has expertise in a wide range of electron microscopy methods and evaluates experimental methodologies and approaches in order to make ultrastructural analyses readily comprehensive to non-EM scientists. The EMRC conducts variety of immuno Electron Microscopy applications and Corrective Light and Electron Microscopy (CLEM) studies between fluorescent microscopy and electron microscopy in SEM and TEM platforms. CLEM allows precise mapping of fluorescent-tagged molecules in the context of functional organizations. The EMRC offers training for negative staining and cryo-EM operations for structural biology. This includes glow discharge, freezing samples with a cryo-plunger, FEI Vitrobot Mark VI, and cryo-EM operation for data collection, which is an essential preceding training for High Resolution Cryo-Electron Microscopes.

The EMRC is generously supported by The Rockefeller University, is open to all University researchers and to non-Rockefeller University researchers, depending on the capacity. The Center is under the direction of Kunihiro Uryu. Ph.D. Dr. Uryu, who obtained his Ph.D. in Anatomy in Nagoya University in Japan, has more than 25 years of experience in anatomy, pathology, cell culture, and protein chemistry in the field of neuroscience. He works with two highly talented Ph.D. specialists. The EMRC is located at the first floor of Rockefeller Research Building on The Rockefeller University campus and encompasses over 2200 square feet lab space.

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 three advanced benchtop analyzers (two BD LSRIIs and one BD LSR-Fortessa) equipped with up to five-lasers (488, 561, 640, 355, 405 nm, and 445 excitation wavelengths) and 18 fluorescence channels. The FCRC also has one basic analyzer, 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 has five analysis computers (two MACs and three PCs) which are loaded with the flow cytometry and office software for data analysis and preparation materials for the publications.

The FCRC, located in approximately 1700 square feet 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 21 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 2500 sequencers, two Illumina NextSeq 500 sequencers, one Illumina MiSeq sequencer, and two realtime PCR systems (Life Technologies QuantStudio 12K flex and Roche LightCycler). The center also provides several accessory instruments for sample quantity and quality validation: Agilent Bioanalyzer, Agilent TapeStation, NanoDrop

spectrophotometer, and Qubit spectrophotometer. 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, the center offers full services for genomic DNA-Seq and RNA-Seq, specializing in preparation of libraries from a very small amount of starting total RNA. Users can also prepare their own libraries and use the center's sequencing-only services. The center offers free consultations on experimental designs, library preparation options, sequencer choice, sequencing depth and coverage, and biological replicates. The center performs initial sequencing data analysis for all users, and can assist with downstream analysis.
The center is staffed by six 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. The center is staffed during regular business hours and is accessible 24/7 with valid RU key cards. The center offers several instruments for use by trained users at no charge.

High-Throughput and Spectroscopy 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 276,560 compounds automated liquid transfer devices, compound databases, and supports a broad diversity of biophysical and biochemical technologies, typically found in early drug discovery programs.
The center is staffed full-time under an open-access model, whereby, once trained, investigators can use the facility 24/7. The center is directed by J. Fraser Glickman who has a Ph.D. in biochemistry and molecular biology from UC Santa Barbara and over two decades of experience in drug discovery and HTS including 16 years working in the pharmaceutical industry. Dr. Glickman has been directing the HTSRC since 2008. Dr. Glickman is readily available for scientific and technical consultations. There are also four research staff associates with a cumulative 30 years of biophysics, biochemistry, cell biology, and assay development expertise, who support the training, assay miniaturization, automation, data processing, liquid handling, instrument QC and maintenance. In collaboration with visiting scientists, the Center has the capacity to support approximately 15 full HTS projects per year, in addition to various pre-HTS and post-HTS activities.

The center occupies 2500 sq. ft. of lab space located on the Rockefeller campus on the Upper East Side of Manhattan in proximity to Weil-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 pipettes and houses a full cell culture facility. Cell dispensing can be done under full laminar flow Biosafety cabinets, and the center is BSL2 compliant.

Small Molecule Libraries- The Rockefeller University Compound Collection currently consists of 276,560 compounds custom selected from a variety of commercial vendors. The purchasing strategy has striven to include all of the well-known forms of compound synthesis and acquisition, including solid-phase pool-and-split, parallel synthesis, individual synthesis, known drugs and their scaffolds, semi-synthetic approaches, diversity-oriented synthesis and natural product isolation. Compounds have been chosen from vendor catalogs based on Tanimoto-based fingerprint clustering, "relaxed" Lipinski guidelines (molecular weight is <700, except for natural products), and more recently, metrics such as Q.E.D. score *(Bickerton et. al.,2012 Nature Chemistry.4:2 , 90-98.)* and Fsp(3) score (*Lovering et. al. 2009. J. Med. Chem. 52. 6752*) in order to select diverse compounds from various clusters with biophysical properties consistent with drug development. We have either flagged or removed reactive substructures, dyes and known frequent hitter scaffolds (Baell et.al. 2010 *J.Med.Chem*.53; 2719-2740). Most of the compounds can be 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. 3000-10,000 new compounds are purchased every year by choosing compounds from commercial databases. The current is described further on the HTSRC website, http://www.rockefeller.edu/htsrc/libraries.

The library itself is stored as 10 to 15 copies of 5 millimolar solutions in DMSO, heat-sealed and barcoded 384-well small-volume polypropylene microplates. 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 unattended 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 15 freeze-thaw cycles.

A Perkin-Elmer Janus Automated Workstation equipped with 384-array nanohead syringes are used for compound dispensing in the 40 nL-400nL range. The system can process 400 plate replications/assay transfers unattended per day. High-throughput cherry picking and serial dilution is accomplished with this system using the 8-channel vari-span which is capable of accurately selecting and arraying 1000 picks of 0.5 microliter of compound solution per day.

Library Quality Control, Annotation and Analysis- Sample integrity of the library is periodically monitored by HPLC-MS of random samples and all re-confirmed hits from screening are routinely tested by HPLC-MS for purity and integrity. We routinely find that 80% of our hits can be confirmed by HPLC-MS upon first analysis in positive ion mode and further negative ion mode analysis finds high purity and integrity in 95% of our samples.

The library has been analyzed for frequent hitting over 20 independent enzyme, cell based and protein-protein interaction assays over the past 4 years and less than 200 compounds (mostly natural products and known drugs) were identified which affect more than one independent assay, suggesting a low frequency of frequent hitters and enzyme aggregators.

Compound “drug-like”metrics such as QED score *(Bickerton et. al.,2012 Nature Chemistry.4:2 , 90-98.)* and Fsp(3) score (*Lovering et. al. 2009. J. Med. Chem. 52. 6752)* have been conducted and benchmarked against two other publicly used libraries, the MLCPN library and the Memorial-Sloan Kettering Cancer Center Library. We have found that the Rockefeller University library compared favorably, with an average Q.E.D. score of 0.667 +/- 0.18, n= 275,453 and an average Fsp(3) score of 0.346 +/- .18. The MLCPN library had an average QED score of 0.652 +/- 0.17 n=333489. Fsp(3) score was 0.300 +/- 0.176. The MSKCC library scored 0.599 +/- 0.186 for QED and 0.321 +/- 0.212 n= 362,565.

The majority of the library has been tested for cytotoxicity using mitochondrial activity marker, Alamar Blue in two different lymphoma cell lines (Toledo and Ly7). 3125 out of the 170,000 compounds tested to date showed cytotoxicity at 10 µM (>40% inhibition) and these compounds have been flagged. A large fraction of the cytotoxic agents came from a collection of diverse natural products from MicroSource Inc. and the marketed anti-cancer drugs.

For removal of artifacts, the HTSRC houses an automated dynamic light scattering system (Wyatt Technologies), an isothermal calorimetry system (Malvern Inc.), a high throughput suface plasmon resonance system (Bio-Rad) and a microscale thermophoresis system (Nanotemper) for conducting biophysical studies on the screening hits to ensure solubility, and suitable stoichiometry and binding affinity. To date, approximately 45 different screens have been performed on the majority of compounds, and these annotations are available for hit selection using the Collaborative Drug Discovery database (https://www.collaborativedrug.com/ CDD, Burlingame California).

General Prioritization of Hits and Follow-up Procedures- The output of our compound screening process are a series of concentration response curves generated from picking from the primary hit list, and the associated HPLC-MS analyses of the sample composition. In many cases, secondary assays, cytotoxicity assays and/or selectivity assays can be performed in parallel so as to eliminate false positives, ensure mechanism of action, or choose the more selective hits. For example, in order to aid in the selection of the best hits for follow-up studies, routine profiling of screening hits is performed using three cell health measurements (ATP production, Membrane Integrity, apoptosis and Nuclear Integrity) on three different cell lines, HepG2 (human liver), SK-N-SH (human neuroblastoma), and MRC5 (human lung).

Additionally hits can be selected by a number of project specific computed criteria (using Pipeline Pilot software) including drug-like characteristics, such as solubility, polar surface area, calculated metabolism, protein binding, Q.E.D. score, fsp3 score, calculated membrane absorption and toxicity.

The center supports procurement of the structurally validated and biologically confirmed hits from the library vendors, and can search the commercial databases for analogs and the further analysis of these in primary and secondary assays in order to find more potent or selective compounds and to gain preliminary knowledge of structure-activity relationships. In addition to the widely available “emolecule” and “molport” databases, a comprehensive in-house vendor database of is maintained. This allows for sub-structure and similarity searching and procurement of over 4 million commercially available compounds for structure activity studies by inventory.

In cases where structural biology efforts are possible, we typically endeavor to obtain structures of the compound-receptor complex, or to model compound-receptor interactions through our structural biology core facility (www.rockefeller.edu/sbrc/).

Informatics- All screening data can be normalized, stored, processed and queried using the *Collaborative Drug Discovery* Database. (https://www.collaborativedrug.com/ CDD, Burlingame California). The database can be accessed by all users over the worldwide web and data remains separated by project. All data can be downloaded into Microsoft Excel, or with structural information such as .sdf or comma delimited smiles formats. Thus, data can be manipulated online or offline, or uploaded into any various software or databases such as PubChem.

Using this CDD database , calculated properties, frequent hitters, cytotoxic compounds and "heatmap" displays are easily viewed. Compound profiles can be determined using cross screen analysis. Statistical values such as Z and Z-prime can readily be calculated. High-throughput concentration-response curve-fitting and classification (Inglese et. al. 2006. PNAS 103:31, 11473) is also performed. Studies of the structure-activity relationships, similarity, sub-structure searches and Bayesian predictions can be accomplished using *Accelrys Pipeline Pilot* software. Licenses for Data Plotting software (*Dotmatics, Vortex*) are also maintained.

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)

Human ORFeome Library- The ORFeome Collaboration (OC) is a group of academic and commercial laboratories formed to develop an unrestricted source of sequence-validated human ORF clones. Using MGC cDNA clones as starting material, the OC has been able to create 16,100 full-length human ORF clones, promoting the goal of providing at least one ORF for each of the human genes. Using Gateway recombinational cloning system in all of the OC ORF clones allows for efficient transfer of the ORFs from one vector to another, providing a broad choice of destination vectors. This ORFeome is available to all HTSRC local users and is compatible with lentiviral expression thereby enabling both targeted experiments and high-throughput screens in diverse cell types.

Instruments- The Center is configured for processing 384-well microplates 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, SPE-Mass Spectrometry and 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. The Center also supports a portfolio of spectroscopic and calorimetric equipment for use in studies of the structure, function and interactions of biological and organic molecules.

Spectroscopy and Biomolecular Interactions- A large portfolio of instruments and their associated expertise are available for analysis of binding kinetics, structure and affinity of protein-small molecule, protein-protein and protein-nucleic acid interactions. The Center supports experimental design, training and guidance in the use of a circular dichroism spectrometer (CD), a surface plasmon resonance (SPR) instruments (Bio-Rad Proteon XPR), a microvolume isothermal calorimeter (GE AutoITC 200), a microscale thermophoresis instrument (Nanotemper MST), a Bruker NMR instrument (600 mHz) with a cryoprobe and a Wyatt Dynapro Dynamic Light Scattering Instrument. These instruments are particularly useful for making sure the integrity, solubility and mechanism of action of small molecule screening hits are confirmed by biophysical analysis.

Microplate Readers with Automated Plate Feeding

Hammmatsu FDSS 6000
Fluorescence Kinetics, calcium flux assays
384 wells in 2-4 minutes

Molecular Devices ImageXpress Micro XL
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.

Biotek Synergy NEO (2)
This is a multi-purpose high-speed microplate reader with dual monochromators which can allow one to dial in the particular wavelengths of interest. This instrument can read AlphaLisa, TR-FRET, and fluorescence polarization assays. It is equipped with in-line injection port which allows for rapid kinetic analyses. 384-well plate/2 minutes

LICOR Odyssey SA
This is an infrared laser scanning plate reader which allows for two channel detection. The system is well suited for ELISA and “In-cell Western” applications, allowing for internal normalization and broad and linear dynamic range for more accurate quantification versus chemiluminescent systems.

Agilent Rapid-fire 365
This is a high throughput mass spectrometry based detection system, ideally suited for difficult to measure enzymes and for measuring cellular metabolites.

Wyatt Dynapro
This is a high-throughput dynamic light scattering instrument. The system can read 384-well plates and is ideally suited for measuring aggregation and solubility (average particle size) of small molecules and biomolecules. We routinely use this to test all of our screening hits as a quality control measure to

ensure that the compounds are soluble at the concentration needed for the assay, and to ensure that there are no compound mediated protein aggregation artifacts. The instrument also can be used for polymerization dynamics.

Perkin-Elmer TriLux2 (Scintillation Proximity and Luminescence)
384 wells in 30-40 minutes scintillation
384 wells in 5 minutes luminescence

Fluidics and Automated Pipetting Workstations

Perkin-Elmer Janus Automated Workstation and Tecan Evo/HiRes NanoServe
Disposable tips or nano head syringes are used for compound dispensing in the 50 nL-50 uL range, 96, 384-well formats. Can process 200 plate replications/transfers or 6000 cherry picks automatically.

TECAN EVO Automated Pipetting Station

Disposable tips are used for compound dispensing in the 500nL-50 uL range, 96, 384-well formats. Can process 200 plate replications/transfers or 6000 cherry picks automatically

Thermo MultiDrop Combi **(2)**
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 loaded 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 microplates. 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 autoclaveable.**

Perkin-Elmer FlexDrop
This is a non-contact, solenoid-valve based plate filler capable of dispensing 0.5 uL-5uL of solution or suspension in 384, or 1536 well formats **with high precision (CV <4%) . 50 plates can be loaded onto the stacker, and loaded with a flask of single cell suspension or enzyme, in 50 minutes.**

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. This instrument can be programmed to perform multi-step ELISA assays with 4 separate reagents and washing

Selected Publications (out of 45)

Koh J, Blobel G. (2015) **Allosteric Regulation in Gating the Central Channel of the Nuclear Pore Complex**. Cell. Jun 4;161(6):1361-73.

Dittmann M, Hoffmann HH, Scull MA, Gilmore RH, Bell KL, Ciancanelli M, Wilson SJ, Crotta S, Yu Y, Flatley B, Xiao JW, Casanova JL, Wack A, Bieniasz PD, Rice CM. (2015) **A serpin shapes the extracellular environment to prevent influenza A virus maturation**. Cell. Feb 12;160(4):631-43

Goglia AG, Delsite R, Luz AN, Shahbazian D, Salem AF, Sundaram RK, Chiaravalli J, Hendrikx PJ, Wilshire JA, Jasin M, Kluger HM, Glickman JF, Powell SN, Bindra RS. (2015) **Identification of novel radiosensitizers in a high-throughput, cell-based screen for DSB repair inhibitors.** Mol Cancer Ther. Feb;14(2):326-42.

Fridy PC, Li Y, Keegan S, Thompson MK, Nudelman I, Scheid JF, Oeffinger M, Nussenzweig MC, Fenyö D, Chait BT, Rout MP. **A robust pipeline for rapid production of versatile nanobody repertoires**.(2014) Nat Methods. Dec;11(12):1253-60.

Ahn HJ, Glickman JF, Poon KL, Zamolodchikov D, Jno-Charles OC, Norris EH, Strickland S. (2014) **A novel Aβ-fibrinogen interaction inhibitor rescues altered thrombosis and cognitive decline in Alzheimer's disease mice.** J Exp Med. May 12. [Epub ahead of print] PubMed PMID: 24821909.

Elissaveta Petrova, Emilie Legué, Jessica Rios-Esteves, Alexandra L. Joyner, Ouathek Ouerfelli, J. Fraser Glickman, and Marilyn D. Resh (2013) **Potent, Specific Inhibitors of Hedgehog Acyltransferase Block Sonic Hedgehog Signaling**. *Nature Chemical Biology*, Apr;9(4):247-9

Lorena Fontan,Chenghua Yang, Venkataraman Kabaleeswaran, Laurent Volpon, Michael J. Osborne, Elena Beltran, Monica Garcia, Leandro Cerchietti, Rita Shaknovich, Shao Ning Yang, Fang Fang, Randy D. Gascoyne Jose Angel Martinez-Climent, J. Fraser Glickman, Katherine Borden, Hao Wu, Ari Melnick. (2012) **MALT1 Small Molecule Inhibitors Specifically Suppress ABC-DLBCL In Vitro and In Vivo** *Cancer Cell* 22, Issue 6, 11 812–824

Gold B, Pingle M, Brickner SJ, Shah N, Roberts J, Rundell M, Bracken WC, Warrier T, Somersan S, Venugopal A, Darby C, Jiang X, Warren JD, Fernandez J, Ouerfelli O, Nuermberger EL, Cunningham-Bussel A, Rath P, Chidawanyika T, Deng H, Realubit R, Glickman JF, Nathan CF. (2012) **Nonsteroidal anti-inflammatory drug sensitizes Mycobacterium tuberculosis to endogenous and exogenous antimicrobials.** *Proc Natl Acad Sci U S A.* Sep 10. [Epub ahead of print] PMID: 23012453

Rujoi, M., Pipalia, N.H., and Maxfield, F.R. (2010) **Cholesterol pathways affected by small molecules that decrease sterol levels in Niemann-Pick type C mutant cells.** *PLoS ONE* 5: e12788. doi:10.1371/journal.pone.0012788.

Antibody and Bioresource Core Facility

The Memorial Sloan Kettering Cancer Center-The Rockefeller University Antibody and Bioresource Center generates custom monoclonal antibodies (MAbs), produces large scale quantities of monoclonal antibodies, distributes cell lines created by both institutions and tests research samples for mycoplasma on a fee for service basis. Grounded in a thorough understanding of MAb technology, the Core's staff provides comprehensive oversight and is 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 facility can produce and or purify up to 4,000 mg of a MAb. 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 two additional full time staff members, occupies about 1,250 square feet of space, and has facilities located on both the MSK and The 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 MSK research laboratories have first priority. The Antibody and Bioresource Core Facility currently supports over 100 research groups at Rockefeller and MSK spanning basic and translational research area.

Precision Fabrication Facility

The Precision Fabrication Facility (PFF) provides access to various fabrication and rapid prototyping tools for qualified users. The shop allows users to design and build their own research tools by providing access to a wide array of prototyping and fabrication equipment, as well as design and fabrication consultation.

The PFF also provides design consultation and fabrication assistance to all University researchers.

Proteomics Resource Center (PRC)

The Proteomics Resource Center is directed by Henrik Molina, Ph.D. who oversees a staff of five scientists. 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 masters most aspects of mass spectrometry based proteomics which includes targeted studies as well as quantitative proteomics based on metabolic labeling (SILAC: *stable isotope labeling with amino acids in cell culture*), chemical labeling , tandem-mass tag technology and absolute quantitation. The Center also offers LC-MS-based small molecule analysis. Very importantly, the Center is a source for help with planning of mass spectrometry based proteomics experiments and the Center have the capability to offer in-depth collaborative data analysis.

The Center operates *state-of-the-art* mass spectrometer equipped with nano and high flow HPLC. 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. A MALDI-TOF mass spectrometer, a Typhoon variable mode imager and HPLC is available for user access.

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.

Selected publications

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(2) Hoshino, A.; Costa-Silva, B.; Shen, T. L.; Rodrigues, G.; Hashimoto, A.; Tesic Mark, M.; Molina, H.; Kohsaka, S.; Di Giannatale, A.; Ceder, S.; Singh, S.; Williams, C.; Soplop, N.; Uryu, K.; Pharmer, L.; King, T.; Bojmar, L.; Davies, A. E.; Ararso, Y.; Zhang, T.; Zhang, H.; Hernandez, J.; Weiss, J. M.; Dumont-Cole, V. D.;

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(3) Lood, R.; Raz, A.; Molina, H.; Euler, C. W.; Fischetti, V. A.: A highly active and negatively charged Streptococcus pyogenes lysin with a rare D-alanyl-L-alanine endopeptidase activity protects mice against streptococcal bacteremia. *Antimicrobial agents and chemotherapy* 2014.

(4) Bunkenborg, J.; Espadas, G.; Molina, H.: Cutting edge proteomics: benchmarking of six commercial trypsins. *J Proteome Res* 2013, *12*, 3631-41.

(5) Maze, I.; Wenderski, W.; Noh, K. M.; Bagot, R. C.; Tzavaras, N.; Purushothaman, I.; Elsasser, S. J.; Guo, Y.; Ionete, C.; Hurd, Y. L.; Tamminga, C. A.; Halene, T.; Farrelly, L.; Soshnev, A. A.; Wen, D.; Rafii, S.; Birtwistle, M. R.; Akbarian, S.; Buchholz, B. A.; Blitzer, R. D.; Nestler, E. J.; Yuan, Z. F.; Garcia, B. A.; Shen, L.; Molina, H.; Allis, C. D.: Critical Role of Histone Turnover in Neuronal Transcription and Plasticity. *Neuron* 2015, *87*, 77-94.

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 a nuclear magnetic resonance spectrometer (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 provides 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, a stereomicroscope (Nikon SMZ18) for crystal tray observations and crystal mounting 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. For protein analysis prior to structural studies the SBRC has a Phastsystem by Pharmacia (now GE) and for analysis/purification of protein samples an Äkta Purifier is available either as a hands-on instrument or as a fee for service by the SBRC staff. The SBRC also offers 20°C incubator space for crystallization trays, incubator shakers and fermenters for protein expression.
The center, generously subsidized by the University, offers consultations about structural information and graphical representations, as well as intellectual contributions at all stages of structure determination. Under special agreements made by the University, researchers have

access to beam line 24ID of the Advanced Photon Source at Argonne National Laboratory and will have direct access to the National Synchrotron Light Source (NSLS-II) at Brookhaven National Laboratory when it becomes available (spring 2016). Coordination of data collection at these facilities and accompanying support is offered.

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.