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.

Bio-Imaging Resource Center
The Frits and Rita Markus 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), two laser scanning confocal microscopes (a Zeiss LSM 780 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), the Celldiscoverer 7 automated live cell imaging system (Zeiss), an iSIM super resolution system (BioVision/Leica), 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 five 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 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 three high end dedicated cryo-electron microscopes. 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 two FEI Titan Krios units which are 300kV TEMs. All 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. The systems are equipped with high brightness field emission guns (FEGs). These FEGs produce coherent electrons, aiding in boosting contrast for data acquisition. One Krios and the Arctica are fitted with Gatan K2 Summit direct electron detectors. The second Krios is equipped with the Gatan K3 camera. These tools are tailored for high throughput data collection of 2D and 3D images. The Center recently commissioned the newly acquired Aquilos Cryo-FIB SEM which will enhance and support tomography studies.

The CEMRC suite is located in the Collaborative Research Center. Priority access to the CEMRC is given to Rockefeller scientists and the University generously subsidizes the cost of operation. The center has a staff of three, including Mark Ebrahim who heads the staff. 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 instruments and competent expertise to support scientists with a broad range of electron microscopy (EM) studies, including sample preparation, transmission and scanning electron microscopy image acquisition and interpretation. Our staff is very experienced in assisting with the design of EM experiments, in choosing the best approach for each project and in the interpretation of results. Basic services include sample preparation for a variety of experimental models which includes but it is not limited to bacteria, yeast, cells in culture, C. elegans, Drosophila, zebrafish and mouse, using conventional chemical fixation, microwave techniques,
high-pressure freezing and freeze-substitution. We offer semi-thin and ultrathin sectioning, staining and EM imaging. Advanced services include immuno-labeling at the EM level, correlative light and electron microscopy techniques and development of protocols for special research needs. Scientists can be trained in the operation of instruments and methodologies or alternatively, EMRC staff can perform the experiments upon request.

The EMRC has two transmission electron microscopes: JEOL JEM1400 Plus, FEI TECNAI 12 BioTwin, and one scanning electron microscope: Zeiss Leo1550. The JEM1400 Plus and Tecnai 12 have automated tomography and montage capabilities. In addition, the JEM1400 plus is equipped for Scanning Transmission Electron Microscopy (STEM). The EMRC also offers a collection of accessory devices; including Edwards Vacuum Evaporator 306A, Leica Sputter Coater ACE600, Leica High Pressure Freezer EMPACT2/Freeze Substitution unit AFS, microwave system (PELCO BioWave), Vitrobot Mark IV and ambient or cryo-Ultramicrotomes.

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 Hilda Amalia Pasolli, Ph.D. Dr. Pasolli, who obtained her Ph.D. in Chemical Sciences at Cordoba National University is Argentina, has more than 25 years of experience in biochemistry, cell and developmental biology and research focus on applying EM to answer scientific questions. The EMRC encompasses over 2200 square feet of lab space on the first floor of the Rockefeller Research 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 and spectral flow cytometers. The FCRC five 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.

For cell sorting, the FCRC at RU is equipped with three BD FACSaria Cell Sorters from BD Biosciences equipped with up to six lasers (488, 561, 640, 355, 405 and 445 nm excitation wavelengths) and 18 fluorescence channels detection and are able to perform high-purity sterile sorts into tubes, 96- and 384-well plates, slides or custom devices at flow rate up to 20,000 events per second. All of the Cell Sorters are placed in the BioBubble Cabinets which allow safe sorting of the RG2 materials. Cell Sorters are operated by FCRC staff only. For analysis purposes, the FCRC at RU is equipped with seven benchtop analyzers, which cover the wide range of researcher’s needs: Amnis ImageStream-X (IMAGE analyzer) is operated by FCRC staff. It 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 great accuracy. The ImageStream-X is able to simultaneously record up to 10-color images with four laser excitations (488, 561, 658 and 405 nm wavelengths). Cytek Aurora (SPECTRAL Analyzer) has four lasers (488, 561, 640 and 405 nm) and 48 fluorescence channels. It has a unique capability of measuring the entire emission spectra of the fluorescent dyes excited by multiple lasers installed on the instrument. The full spectrum capture enables the use of the novel un-mixing algorithms for the further data analysis. Currently, at least 24 different fluorescent labels could be resolved, even when multiple markers are co-expressed on the same cell. Five benchtop alignment-free multipurpose analyzers: four Advanced Analyzers (BD LSRII-1, BD LSRII-2, BD LSR-Fortessa, and ThermoFisher Attune NXT) and one Basic Analyzer (BD Accuri® C6). Diverse laser/detector configurations on these instruments
allow for analysis of cell samples stained with 488 nm, 640 nm, 355 nm (UV), 405 nm, 445 nm and 561 nm excited dyes. A number of applications, including the multicolor analysis of cell phenotype, gene expression, cell cycle, and others may be performed.

After orientation training (Virtual Classroom, "Beyond the Basics" Class and Instrument Hands-On Training), Cytek Aurora, BD LSRII-1, BD LSRII-2, BD LSR-Fortessa, ThermoFisher Attune NXT, and BD Accuri C6 are available for use directly by the investigators.

The FCRC has six analysis computers (two MACs and four 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 2100 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 25 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 an Illumina NovaSeq 6000 sequencer, two Illumina NextSeq 500 sequencers, one Illumina MiSeq sequencer, a 10X Genomics Chromium Single Cell System, and a Life Technologies QuantStudio 12K flex realtime PCR system. The center also provides several accessory instruments for sample quantity and quality validation: Agilent Bioanalyzer, Agilent TapeStation, NanoDrop spectrophotometer, and Qubit fluorometer.

For the next-generation 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 Ph.D. 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.

The Rockefeller University High-Throughput and Spectroscopy Resource Center

(http://www.rockefeller.edu/htsrc)

SUMMARY
High Throughput Screening (HTS) is an important step in the discovery of new medicines and involves the miniaturization and automation of bioassays so that millions of compounds can be tested. The High Throughput and Spectroscopy Resource Center (HTSRC) supports the faculty and students in improving
the efficiency of their bioassays, identifying compounds and genetic modulators of function, and in utilizing core technologies typically applied to analysis of molecular recognition.

The center is configured for processing 384-well microplates using automated changeable tip dispensers, non-contact dispensers, and pin-tools. 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, In-Cell Western, SPE-Mass Spectrometry and bioluminescence, scintillation proximity and high-content microscopy. Assay targets can include ion channels, receptors, enzymes, protein interactions, signaling pathways and cellular processes.

LABORATORY AND STAFF
The 2800 sq. ft. laboratory space 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 University of California, Santa Barbara and over two decades of experience in drug discovery and HTS including 16 years working in the pharmaceutical and biotech industries. 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 specialists 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. With the help of the students and trainees, the center has the capacity to support approximately 15 full HTS projects per year, in addition to assay development, orthogonal assay testing and lead optimization activities.

The lab space is equipped with 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 383,000 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 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 the frequent hitters, which represent less than 0.25% of the screening compounds have been flagged. Most of the compounds can be reordered 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 current library is described further on the HTSRC website, http://www.rockefeller.edu/htsrc/libraries. Random Samples and hits are periodically analyzed by HPLC-MS to verify compound purity and integrity, as well as proving that the lab informatics system of compound assignment is correct.

The library compounds are 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°C in REVCO freezers with emergency power and the two working copies are stored at -20°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, a NanoServe plate carousel and barcode reader. Cellario scheduling software allows the system to deliver large numbers of library plates in short order with minimal heat-cooling cycles. The "working" copies of the library are used for a maximum of 15 freeze-thaw cycles.

Fragment-Based Screening
The HTSRC maintains a fragment-based screening platform based on 2184 fragments purchased from LifeChemicals, with measured high solubility in aqueous media. Each fragment represents a cluster, from which analogs of hits can be purchased for initial S.A.R. studies. Our basic platform includes 240 uM-1mM primary screening using DSF, followed by hit confirmation using either MST, SPR or ITC. Functional assay readouts are also amenable to this form of compound screening.

Drug Repurposing and Annotated Compounds
The HTSRC maintains a collection of 5900 known drugs, clinically used compounds and annotated tool compounds procured from various vendors, and includes the NIH clinical collection, the Prestwick Collection and the Selleck Collection of known drugs and annotated kinase inhibitors.

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.

Nuisance Compounds and Frequent Hitters
The library has been analyzed for frequent hitting over 20 independent enzyme, cell based and protein-protein interaction assays over the past 8 years and less than 300 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.

Benchmarking
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 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 HTSRC compound library was benchmarked by the National Institutes of Health (NIH)/National Center for Advancing Translational Sciences cheminformatics group. Over 95% of the compounds are diverse, high quality, and overlapped in similarity and quality metrics with the NIH collection. Only 350 compounds out of 380,000 were shown to be significantly different.

Annotation
Most of the library has been tested for cytotoxicity using the cell titer-glo assay. 4125 out of 320,000 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.

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). All compounds which have publicly available annotation contain hyperlinks to outside data from ChemSpider and other miscellaneous public information.

GENERAL PRIORITIZATION OF HITS AND FOLLOW-UP PROCEDURES
The output of our compound screening process is a series of concentration response curves selected 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 to eliminate false positives, ensure mechanism of action, or choose the more selective hits.

Cheminformatic analysis of the screening data involves six basic activities. 1) selecting hits based on standard statistically significant cutoff criteria normalized against controls and subsequently performing concentration response experiments to rank compounds based on potency and curve fitting 2) similarity and sub-structure searching public databases for publications, patents and other miscellaneous annotation about the active compounds. These include patents and clinical trials and pharmacological data using Elsevier Reaxys, Probes & Drugs Portal, ChemSpider and PubChem. This information is used to decide which compounds may be more useful or informative to pursue and to assess any off-target effects 3) similarity and substructure searching our internal CDD database to see if the compound was active against any prior targets or phenotypic assays screened. This information allows us to choose compounds with little potential for side-effects and to possibly understand mechanism of action in phenotypic studies. 4) Searching for similar analogs and scaffolds that were screened to develop understanding of any structure-activity relationships derived from the primary screen. 5) Searching Molport and contacting the library vendor to provide re-supply of compound powders and to provide focused libraries or sets of analogs for further screening in order to develop and define structure-activity relationships. 6) removal of any nuisance compounds based on REOS, QED and PAINS flags. Based on the information collected, decisions can be made regarding which scaffolds to focus on. 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, or blood-brain barrier permeability calculations.

The center supports procurement of the structurally validated and biologically confirmed hits from the library vendors, and can search the commercial databases for congeners 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 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/) or through collaborations with X-ray crystallographers.

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 over the internet by all users and the 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 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 for data visualization, clustering and publication illustrations.

INSTRUMENTS

Spectroscopy and Biomolecular Interactions
An excellent 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 an Applied Photophysics Chirascan circular dichroism spectrometer (CD), a high-throughput surface plasmon resonance (SPR) instrument (Bio-Rad Proteon XPR), a microvolume isothermal calorimeter (Malvern AutoITC 200), a high throughput microscale thermophoresis instrument (Nanotemper Dianthus NT 23 PicoDuo MST), Tycho NT6 and BioRad CFX thermal melt analysis instruments. For measurement of compound integrity, purity and solubility, the center supports a Thermo Fourier-Transfer Infrared Spectrometer, a Wyatt Dynapro Dynamic Light Scattering Instrument and an Agilent HPLC-TOF mass spectrometer.

Microplate Readers with Automated Plate Feeding

Hamamatsu FDSS 6000
This is a fluorescence imaging plate reader which utilizes a CCD camara combined with internal liquid handling and plate magazine to perform calcium flux and ion channel assays in a 384 wells format in 2-4 minutes per plate.

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. One of our systems is equipped with a Biotek Rapid-Stak automated microplate magazine.

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. Our system is fed by a Biotek Rapid-Stak automated plate magazine.

Perkin-Elmer TriLux2 (Scintillation Proximity and Luminescence)
This is a high-throughput scintillation/luminescence counter that can handle 384 wells in 30-40 minutes scintillation or 384 wells in 5 minutes for luminescence. It has a 15-MTP magazine for autoloading.
**High-Content Screening and Instrumentation**

*Molecular Devices ImageXpress Micro XL*
This is a high-throughput Fluorescence Microscopic Imaging system, with laser-based autofocus, automated microplate handling, and automated image segmentation software. **it is capable of handling 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.

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

*SeaHorse XF96 Extracellular Flux Analyzer*
This instrument is useful in studying cell metabolism, as it can kinetically measure the dissolved oxygen and pH of the cell media surrounding cells cultured in 96-well plates. pH changes and oxygen consumption correlate with anaerobic and aerobic metabolism. The system can be used for studying the effects of compounds on metabolism. It is additionally useful for studying adipose function and differentiation and other aspects of mitochondrial and glycolytic function in living cells and isolated mitochondria.

**Fluidics and Automated Pipetting Workstations**

*Perkin-Elmer Janus Automated Pipetting Workstation*
This system uses disposable tips, nano syringes or pin-tools for dispensing in the 50 nL-50 uL range, 96 or 384-well formats. It can process 200 plate replications/transfers or 6000 cherry picks unattended. A *Perkin-Elmer Janus Automated Workstation* equipped with 384-well pin tools is used for compound dispensing in the 10 nL-100nL range. The system can process 200 plate replications/assay transfers unattended per day. High-throughput cherry-picking and serial dilution is accomplished with this system using the 8-channel Varispan which is capable of accurately selecting and arraying 500 picks of 0.5 microliter of compound solution per day.

*TECAN EVO Automated Pipetting Workstation*
This system uses disposable tips or pin tools are for compound dispensing in the 500nL-50 uL range, 96, 384-well formats. This is used as a walk-up instrument for custom library formatting, genetic screens or any task where users need to do custom high throughput pipetting.

*Thermo MultiDrop Combi (2)*
These are non-contact, peristaltic microplate fillers 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 autoclavable.

*Biotek EL406 Microtiter Plate Washer with BioStak automated microplate magazine.*
This precision instrument washes a 96/384 well plates in 30 seconds or less, with precise control of flow
rates and tip distances. It can be programmed to perform multi-step ELISA assays with 4 separate reagents for washing.

RECENT HTSRC-SUPPORTED PUBLICATIONS (OUT OF 78)


**Precision Instrumentation Technologies (PIT)**

The Precision Instrumentation Technologies facility provides access to various fabrication and rapid prototyping tools, including a state of the art Hermle 5-axis CNC mill, for qualified users. The PIT 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 support by expert engineers and a master machinist for full service design and fabrication.

**Proteomics Resource Center (PRC)**

The Proteomics Resource Center is directed by Henrik Molina, Ph.D. who oversees a staff of six scientists. Dr. Molina’s experience is based on more than two decades working in most aspects of mass spectrometry based proteomics, 90+ publications, 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 Proteomics Resource Center at The Rockefeller University masters most aspects of mass spectrometry based proteomics which includes, *de novo* sequencing (1), targeted studies (2), quantitative proteomics profiling based on label free quantitation (3) as well as metabolic labelled samples (SILAC) (4), chemical labeling (5), tandem-mass tag technology (6) (7), absolute quantitation (8) and global post translational analysis (9, 10). The Center also offers LC-MS based analysis of small molecules (11), polar metabolites (12) and lipids (13). Very importantly, the Center is a source for help with planning of mass spectrometry based experiments and the Center have the capability to offer in-depth collaborative analysis. Also, the PRC is operated in a *boutique* style; encouraging the scientists of the Center to work closely with users to create a tailored approach to fit the many unique questions that can be answered by analytical mass spectrometry.

The PRC are equipped with Orbitrap type mass spectrometers (high resolution/high mass accuracy), operates a triple quadrupole and can separate analytes using both nano and high flow liquid chromatography. The Center is equipped with multiple high performance servers for data analysis. 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.

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 Center works with close to 200 users yearly and logs more than 5000 hours of mass spec analysis per year.


The Structural Biology Resource Center (SBRC) located in RRB140 is an expert resource for protein expression and purification and structural biology analysis by x-ray and beamline studies. The mission of the SBRC is to offer the expertise and tools that are the bread and butter of Structural Biology, to the benefit of research at The Rockefeller University.

Protein expression in various systems, such as e. coli or insect cells can be carried out in the center with assistance in experimental design and troubleshooting.

For protein purification and analysis the SBRC has a Phastsystem by Pharmacia (now GE), Äkta Purifier and Bio-Rad NGCQuest10 chromatographic systems for ion exchange, affinity and size-exclusion, and an SEC-MALS by Wyatt for analysis of protein’s absolute molecular weight and oligomeric state. These instruments are available either hands-on or fee-for-service by the SBRC staff.

For crystallization the center houses the option of UV fluorescence imaging of crystallization experiments. This technique allows the experimenter to determine whether very tiny crystals are in fact protein and not salt or other undesired components. This microscope is made by JanScientific and is capable of identifying Tryptophan-containing protein crystals by excitation in the deep UV (280nm) and observing the emission at 350 nm. Also available is a stereomicroscope, Nikon SMZ18, for crystal tray observations and crystal mounting. Robotic liquid handling is offered at the center, to facilitate in the task of setting up hundreds to thousands of crystallization conditions. The Formulator by Formulatrix prepares trays from stock solutions, reducing preparation time from days to minutes and the Phoenix by Art Robbins can dispense the reservoirs from trays made by Formulator (or obtained commercially) to individual trays and create the protein/precipitant drops using any 96-well crystallization tray.

The Center provides training and expert guidance for researchers requiring purified proteins or undertaking crystallographic structure determination. The Center is also the RU liaison to the Advanced Photon Source and Brookhaven National Labs NSLS synchrotron X-ray PRT beam lines.

The SBRC offers:

- Assistance with experimental design in: crystallography, protein expression or purification
- Individualized instruction and training on the instruments
- Consultation on structural information and data analysis
- Experimental troubleshooting
- Maintenance of the instruments
- HKL2000 support
- Synchrotron coordinator

The mission of the SBRC is to offer the most productive structural biology facility through high quality, reliability and open dialog to the benefit of research at The Rockefeller University.

References:

