Weill Cornell Medicine

Core Laboratories Center

Abby & Howard P. Milstein Chemistry Core Facility

ABSTRACT

The WCM CLC Milstein Chemistry Core Facility provides state-of-the-art chemical synthesis and analysis resources and services, and expertise in their applications, to the WCM community and to outside investigators. Services include (1) *chemical synthesis* for compounds that are not readily available, for assay development tools and reagents, for compounds for *in vitro* and *in vivo* assays, for large-scale chemical synthesis of compounds, and for confirmatory synthesis of compounds; and (2) *chemical analysis* for structure-activity relationship studies on pharmacophores, for structure determination of unknown molecular entities, for chemical analysis of reaction mixtures, cell extracts and bioassays, and for molecular modeling and *in silico* screening. The core facility provides consultation on project design and data analysis, and offers seminars, training and educational workshops.

OVERVIEW

History: The Abby and Howard P. Milstein Chemistry Core Facility (MCCF) was founded in 2006 with generous support from the Howard and Abby Milstein Foundation. The core became part of the WCM Core Laboratories Center (CLC) in 2015.

Location: The Milstein Chemistry Core Facility is located on the 16th floor of the Belfer Research Building at 413 East 69th Street, New York, NY. The core is near both the Tri-Institutional Therapeutics Discovery Institute (Tri-I TDI) and the WCM Molecular Imaging Innovations Institute (MI3). This facilitates productive sharing of reagents, instruments, knowledge and expertise, and allows for collaborative interactions with researchers interested in drug discovery and molecular imaging.

WCM Core Laboratories Center (CLC): The WCM CLC was established in 2015. In addition to the chemistry core, the CLC includes core facilities that offer resources and services in genomics and epigenomics, proteomics and metabolomics, NMR, flow cytometry, imaging (including optical and electron microscopy and high content screening) CBIC (MRI, PET/CT, and ultrasound), biorepository, bioinformatics, and advanced technology assessment.

Instruments: Major equipment includes UPLC/MS, preparative HPLC/MS, preparative HPLC, GC/MS, RapidFire high-throughput MS, IR spectrometer, automated microwave synthesizer, and automated chromatography.

Chemical synthesis: Services include synthesis of single compounds that are known in the literature, or have been obtained from a chemical screen, and synthesis of compounds with unknown utility, but are predicted to have desirable properties.

Chemical analysis: Services include identification of unknown and/or known molecular entities, evaluation of and/or purification of chemical reaction mixtures, evaluation of extracts from cellular assays, analysis of biological extracts, serum, or plasma.

Consultation: Consultation on synthetic, medicinal, and analytical chemistry applications, including: project design, molecular modeling, *in silico* library screening, sample preparation, data collection methods, and data analysis.

Administration: The Milstein Chemistry Core Facility is administered by the Weill Cornell Medicine (WCM) Core Laboratories Center (CLC).

Open to all: The resources and services of the Milstein Chemistry Core Facility are open to all investigators at Weill Cornell Medicine, Cornell University and Cornell-affiliated institutions. The facility also provides services to external investigators at both academic institutions and commercial enterprises.

RESOURCES

The core's resources include four chemical fume hoods, a CEM-Discover microwave irradiator, with 48-position autosampler, a Labconco FreeZone Plus 6 liter lyophilizer, as well as a full array of analytical and preparative scale instrumentation, including: Waters ACQUITY SQD UPLC/MS (PDA, fluorescence, and mass detection, up to 2000 amu); Varian preparative HPLC with PDA detection; Varian 4000 GC-MS (internal, external and hybrid ionization); Bruker TENSOR 27 series FT-IR spectrometer equipped with a Diamond ATR; and four ISCO CombiFlash Rf automated chromatography units.

The core has access to resources in the Tri-I TDI, including: Waters ACQUITY I-Class UPLC/MS (PDA, fluorescence, and mass detection, up to 3000 amu); Waters ACQUITY H-Class UPLC/MS (PDA, and mass detection, up to 1250 amu); and Waters Autopure prep HPLC/MS (PDA, evaporative light scattering, and mass detection, up to 3000 amu).

The core also has access to an Agilent RapidFire high-throughput MS system located in the nearby WCM CLC/MCC Proteomics and Metabolomics Core Facility, and to the NMR instruments located in the nearby WCM CLC NMR Core Facility.

Services

Chemical Synthesis

- Chemical synthesis of compounds that are not readily available or are prohibitively expensive from external vendors.
- Synthesis of assay development tools and reagents including: fluorescently labeled compounds; affinity labeled compounds; cross linker-tethered molecules; labeled (^{13}C, ^{2}H, ^{15}N) and radiolabeled (^{3}H, ^{14}C, ^{32}P, ^{125}I, etc.) compounds for preclinical and clinical pharmacological studies, which cannot be directly addressed by the WCM CLC Citigroup Biomedical Imaging Center (CBIC).
- Synthesis of compounds in quantities that allow for *in vitro* and *in vivo* assays, and follow-up assays, when warranted.
- Large-scale chemical synthesis of compounds with demonstrated biological activity in primary assays, to provide material for further investigation.
- Confirmatory synthesis of compounds identified *via* high-throughput screening.
- Structure-activity relationship (SAR) studies on validated pharmacophores in order to optimize targeting, specificity, and bioavailability, while minimizing toxicity.

Analytical Chemistry

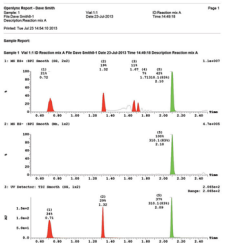
- Structure determination of known and unknown molecular entities.
- Detailed chemical analysis of biological extracts, and bioassays.
- Evaluation of and/or purification of complex reaction mixtures.

Data Analysis and Project Development

Molecular modeling and *in silico* screening using a variety of computational tools, including the Schrödinger software suite and Chemical Computing Group's Molecular Operating Environment (MOE).

Ultra-Performance Liquid Chromatography / Mass Spectrometry (UPLC/MS)

- Confirm the identity and purity of synthesized products, accurately and quickly, using the ACQUITY UPLC system.
- Detection modes include UV/Vis, fluorescence, and electrospray mass spectrometry (up to 3000 m/z).
- Low dispersion design allows complex separations to be accelerated without compromising chromatographic performance.
- Low carryover with the choice of sample manager design (Fixed Loop or Flow-Through-Needle), complementing MS sensitivity and extending linear dynamic range of MS and other UPLC detectors.
- Maximized peak capacity to enhance the sensitivity of MS detector by fully resolving analytes from background interferences.



Typical open access report using the ACQUITY UPLC I-Class System with ACQUITY SQD2 Detector. The highlighted area shows how the ACQUITY SQD2 Detector confirms that the required product (310 Da) has been successfully synthesized while the purity is determined from the ACQUITY UPLC PDA Detector.

RapidFire LC/MS

- Enables direct, enzymatic detection of native analytes without the use of surrogates, radioactivity, coupled assays, or indirect measurements.
- Provides fast results, with cycle times as fast as 8 sec per sample for drug discovery applications and 10 sec per sample for clinical research applications.
- Increases analysis capacity with automated 63 plate handling and 60 hours (weekend-long) unattended operation for 20,000+ injections.
- Increases productivity and efficiency through time-saving, automated method development routines and solvent switching for multiple different assays in a single batch.
- Paired with an Agilent 6495 triple quadrupole mass spectrometer for high levels of sensitivity, precision, and accuracy

Gas Chromatography Mass Spectrometry (GC-MS)

- Maximizes compound identification and quantitation with a wide choice of MS and MS/MS modes.
- Routine, high performance analysis with both internal and external ionization configurations.
- High levels of selectivity and sensitivity for trace analysis with hybrid CI, both positive and negative.
- Three easily switchable ionization configurations: internal ionization (II), external ionization (EI) or hybrid chemical ionization (CI).

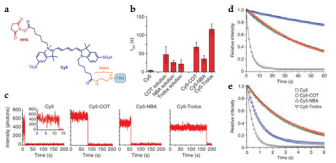
Ultra-Performance Liquid Chromatography / Mass Spectrometry (UPLC/MS)

- Preparative-scale chromatography plays a critical role in applications where compounds must be synthesized, identified, isolated, purified, characterized, screened, and tested. The Waters AutoPure Prep HPLC/MS system offers scalable prep configurations, including the specificity of mass-directed purification, the inclusiveness of a UV/Vis system, and the universality of an evaporative light-scattering detector.
- The system provides the flexibility of using high-throughput parallel runs for selective mass-directed fraction collection from hundreds of samples. With this system, samples can run 24/7 with consistency.
- Versatile solutions are available that are capable of purifying micrograms to multigram quantities automatically – hundreds of samples, or just a few.
- Flexible configurations enable easy scale-up from analytical to preparative chromatography.
- The FractionLynx Application Manager automates the collection of detected fractions, tracks samples and fractions, and then presents the data in an easy-to-view format. This software can trigger collection using a variety of detector signals, including UV/visible, evaporative light scattering (ELS), MS, and analog.

Signature Projects

Development of Fluorophores with Enhanced Photostability

Working in collaboration with Dr. Scott Blanchard's group in the Department of Physiology and Biophysics, we helped to develop a new class of dramatically enhanced fluorophores that exhibit remarkable photostability. These fluorophores have been used extensively by the Blanchard lab and others, in fluorescence-based applications that demand long-lived, non-blinking fluorescence emission.



(a) General schematic for enhanced dyes containing an internal triplet-state quencher (TSQ). (b) Average dwell time for the on state (t_{on}) with individual TSQs in solution. (c) Cy5 fluorescence under direct excitation with and without a TSQ directly linked. (d, e) Photostability of Cy5- and Cy5-TSQ-conjugated DNA duplexes surface-immobilized at a saturating density and in the presence (d) or absence (e) of an oxygen scavenging system.

Altman, R. B.; Terry, D. S.; Zhou, Z.; Zheng, Q.; Geggier, P.; Kolster, R. A.; Zhao, Y.; Javitch, J. A.; Warren, J. D.; Blanchard, S. C. Nat. Methods 2012, 9, 68

Selective Inhibition of the Human Immunoproteasome over the Constitutive Proteasome

Working in collaboration with Drs. Carl Nathan and Gang Lin in the Department of Microbiology & Immunology, we synthesized a focused library of oxathiazolones and found several highly active inhibitors for the human immunoproteasome LMP7 (b5i) subunit over the constitutive proteasome, some of which displayed up to 4700-fold selectivity. Furthermore, these compounds were found to be cell-permeable, and inhibit proteasomes inside the cell.

R	Hu20S β5i k_{inact} x 10^2 (s^{-1})	Hu20S β5i K_I (μM)	Hu20S β5i k_{inact}/K_I ($M^{-1}s^{-1}$)	Hu20S β5c* k_{inact}/K_I ($M^{-1}s^{-1}$)	Ratio
	0.77	0.76	1012.2	10.1	100
	0.93	7.9	118	14.8	8
	0.38	0.42	913.2	10.3	89
	0.26	1.1	225	0.13	1730
	1.54	1.4	1093	0.23	4750

***The plots of k_{obs} vs [I] for hu c-20S were linear. Individual k_{inact} and K_I values cannot be derived; instead k_{inact}/K_I values were derived from the slopes of the plots.**

Fan, H.; Angelo, N. G.; Warren, J. D.; Nathan, C. F.; Lin, G. ACS Med. Chem. Lett. 2014, 5, 405.

Consultation, Workshops and Training

Consultation on project design and data analysis.

Educational workshops and hands-on training.

Seminars on emerging analytical and synthetic chemistry methods, technologies and applications.

Coordinated project design consultation and data analysis support available with the CLC NMR, proteomics and metabolomics, genomics and epigenomics, flow cytometry, imaging (including optical microscopy, multiphoton microscopy, electron microscopy, high content screening, MRI, PET/CT, and ultrasound), biorepository, bioinformatics, and advanced technology assessment core facilities.

Contact Information

Abby & Howard P. Milstein Chemistry Core Facility

J. David Warren, Ph.D., Director
jdwarren@med.cornell.edu

http://corefacilities.weill.cornell.edu/syn_core

For questions about the WCM Core Laboratories Center, please contact George Grillis at ggg2016@med.cornell.edu