# EQUIPMENT

**Program in Chemical Biology (PCB)**

# *Synthetic chemistry.* The Program in Chemical Biology houses a shared resource for synthetic chemistry led by Dr. Smith (Associate Director). The PCB chemistry team is currently staffed by a full-time Ph.D. chemist, Dr. Rob Keyes, who was recruited from the pharmaceutical industry with experience at Abbot Labs and Novartis, and three postdoctoral fellows from the Smith, Marchant (Chair of Cell Biology), and McCorvy (Cell Biology) laboratories. An additional Bachelor’s/Master’s level chemist is being actively recruited to support PCB collaborative projects. The central laboratory includes four 7-ft fume hoods and one 4-ft fume hood. Due to the exceptional productivity of the PCB, we recently received an institutional commitment to relocate to a dedicated space and double the hood capacity of the PCB synthetic chemistry laboratory. The four 7-ft hoods are equipped with custom-designed dual-barreled Schlenk lines for vacuum and water/oxygen-sensitive work and IKA RCT basic hotplate/stirrers (2/hood). Each hood has a dedicated Edwards RV8 direct-drive vacuum pump for the Schlenk line and a Lauda ECO RE 420S recirculating water bath for reflux condensers and other applications. The laboratory also has various belt-driven high vacuum pumps for distillation, drying of compounds, and other applications and a syringe pump for reproducible addition of reagents. The laboratory houses an IKA RV8 rotary evaporator system with an IKA MVP10 vacuum pump and an IKA RC2 recirculating water bath. Complementing this is a Büchi RE-111 rotary evaporator with a KNF Neuberger Labport chemical-resistant diaphragm pump and a Büchi RE-110 rotary evaporator with a Welch Dryfast pump. The laboratory contains an MBraun SPS-800 solvent drying system for water-sensitive work that houses five solvents and a Mettler-Toledo Karl Fischer titration instrument to monitor water content. The laboratory has an MBraun UNI-Lab Pro glovebox with oxygen and water sensors and an attached freezer for oxygen-sensitive work and storing sensitive compounds. The laboratory has an Advion LRMS connected to a PlateExpress for mass spectrometry analysis of TLC plates for reaction analyses (purchased with NIGMS Administrative Supplement for Equipment R35 GM128840-02S1). The laboratory has three Biotage Isolera One Accelerated Chromatographic Isolation systems for rapid normal-phase purification, one equipped with a Dalton 2000 LRMS. These mass spectrometers have interchangeable APCI and ESI sources and direct-injection capabilities for reaction monitoring, compound identification, and mass-directed flash chromatography. The laboratory has an Advion puriFlash 5.250 Preparative HPLC for rapid reverse-phase purification at preparative scales. The laboratory houses a Biotage V10-Touch evaporation system that allows rapid evaporation of aqueous solutions and high-boiling organic solvents (DMSO, DMF, NMP, etc.). The V10 system includes a vial carousel and a Gilson fraction collector that can be queued for automated collection and concentration of purified fractions (HPLC/Isolera) into separate vials. The laboratory includes a Jasco Polarimeter to analyze the optical rotation of chiral molecules. The laboratory contains a Biotage SPWave microwave system with an 8-reaction autosampler and a semi-automated peptide synthesis package for accelerated synthesis and discovery.

***Small molecule (fragment) screening.***The Program in Chemical Biology also provides access to resources, protocols, and support for nuclear magnetic resonance (NMR), microscale thermophoresis (MST), and surface plasmon resonance (SPR) screening of chemical fragment libraries and other small molecules. Chemical fragments and other small molecules purchased or synthesized as potential ligands are given a unique ID and entered into a cheminformatic database, which tracks compound inventories and locations. Protein screening targets are organized in an Airtable online database that collates NMR and other biophysical assay screening results and *in vitro* efficacy and potency for multiple targets. The current fragment collection includes libraries purchased from Maybridge, TargetMol, and Enamine, totaling more than 2000 unique compounds. The LEAP Technologies PAL liquid handling system enables programmed sample preparation for small molecule ligand screening. For NMR screening, the SampleJet robot facilitates the automated screening of compounds in 96-tube racks. We devised a novel scoring metric for improved hit detection from NMR spectra—difference intensity analysis (DIA)—that sums all positive and negative intensities from 2D difference spectra. Applying DIA quickly discriminates potential ligands from compounds inducing protein aggregation and other nonspecific effects. Python scripts were developed in-house to expedite the processing of NMR data, and the TitrView module of nmrDraw streamlines the analysis. For MST and SPR screening, a Nanotemper NT.Automated instrument coupled to a Hamilton Starlet liquid handling robot was recently obtained through S10 OD025036 (PI, Dr. Blake Hill, Professor of Biochemistry). For SPR screening, a BIAcore S200 SPR instrument with increased sensitivity necessary for fragment screening was recently obtained through S10 OD028640 (PI, Dr. Nancy Dahms, Professor of Biochemistry. Together, these instruments enable the highly automated screening of thousands of compounds per week.

**Structural Genomics Unit (SGU)**

The SGU laboratory (Dr. Volkman, Director; Dr. Smith, Associate Director), located in the MCW Mellowes Center for Genomic Sciences & Precision Medicine, is equipped with all necessary instrumentation for high-throughput recombinant protein expression and purification from *E. coli*. Protein targets, individual preps, and plasmids are given a unique ID and entered into an Airtable online database. This system facilitates collaborative applications and databases hyperlinked to LabArchives electronic research notebook entries within the SGU team. For protein expression, the laboratory contains two Thermo Scientific MaxQ 800 stackable incubator shakers capable of cooling and heating. For automated protein purification, the laboratory houses a Promega Maxwell-16 robot for automated, parallel purification of up to 16 distinct proteins using immobilized metal affinity chromatography and a GenScript AmMag SA Plus protein purification robot, capable of 12-channel purification from cell pellet or lysate to purified protein in 40 minutes. For additional protein purification, the laboratory contains an ÅKTA pure FPLC. The FPLC is housed in a Lab Research Products refrigerated cabinet, includes an F9-R fraction collector, and is controlled by an HP computer. To determine protein identity and purity, the laboratory includes a Bio-Rad PowerPac Basic power supply for SDS-PAGE analyses. The laboratory also includes an Eppendorf 5810R refrigerated tabletop centrifuge, a Labnet HERMLE MR-2 tabletop centrifuge, a Scientific Industries Vortex-Genie, an Eppendorf pipet set, a USA Scientific 2D Rocker, a Welbilt 4 °C refrigerator, a Lab Research Products -20 °C freezer, and a Thermo Electron Corporation Forma ULT -80 °C freezer.

# Program in Chemical Biology Shared Equipment

***Biomolecular Nuclear Magnetic Resonance facility*.**The facility provides routine 1D and 2D NMR service, consultation, and collaborative assistance with the acquisition and analysis of multidimensional, multinuclear protein NMR spectra. The facility houses four Bruker NMR spectrometers (one 500 MHz, two 600 MHz, and one 800 MHz) operated and maintained by Dr. Francis Peterson (Professor of Biochemistry, NMR facility manager) in a dedicated 2,500 ft2 facility. Each NMR instrument is equipped with a 1H/15N/13C cryoprobe for ultra-high sensitivity in biomolecular applications. The 500 and 800 MHz instruments also have 19F detection, and the 800 MHz cryoprobe is equipped with three-axis XYZ pulsed-field gradients. The 600 and 800 MHz instruments are fitted with SampleJet robots for automated NMR acquisition of samples in 96-tube racks. One 600 MHz instrument is configured with a 1.7mm 1H/15N/13C cryoprobe (~50 µL sample size). As such, this 600 MHz instrument is dedicated to chemical fragment screening and other automated multi-sample/high-throughput NMR applications. A PAL LEAP liquid handling robot mixes and loads NMR samples in a 96-tube rack format. UPS power supplies and building generator backup systems protect all NMR systems. The NMR suite is maintained at 68 ± 1 °F with a dedicated, high-turnover air handling system. Also, an automated switching device cools helium compressors serving the cryoprobe systems by closed-loop heat exchangers coupled to either plant-chilled water or domestic water systems. A lab bench equipped for routine NMR sample preparation, adjustment, and storage (refrigerator/freezer, pH meter with NMR tube probe, microcentrifuge, micropipettes, buffers, etc.) is located within the NMR suite.

***Shared biophysical instrumentation*.**The Program in Chemical Biology helps maintain BIAcore 3000 and S200 SPR instruments, Molecular Devices Flexstation 3 microplate readers (2) capable of fluorescence polarization measurements, and a Jasco J-1500 circular dichroism spectropolarimeter. The Program in Chemical Biology also houses a Nanotemper Prometheus NT.48 differential scanning fluorimetry (DSF) instrument, and Monolith NT.115 and NT.Automated microscale thermophoresis (MST) instruments for measuring protein thermostability and binding, respectively. Specifically, the Nanotemper Prometheus NT.48 is a 48-channel nanoDSF instrument that measures the thermal unfolding of proteins by DSF and provides melting temperature determinations and other thermodynamic parameters. The Monolith NT.115 instrument is a 16-channel instrument equipped with nanoBlue/picoRed channels for quantitative analysis of protein-protein and protein-ligand binding reactions in a concentration-dependent manner, providing a *K*d value in ~45 min. The NT.Automated MST instrument is configured with Pico-red (1 pM to mM affinity range), nano blue (1 nM to mM affinity range), and LabelFree (UV) channels that allow measurement of pM-mM affinities using fluorescently-labeled proteins and µM-mM affinities using intrinsic fluorescence. The NT.Automated is paired with a Hamilton STARlet liquid handling robot capable of making compound dilutions and allowing automation of sample preparation with MST data collection.

**MCW Shared Equipment within Research Centers**

***Mellowes Center for Genomic Sciences & Precision Medicine.***The Mellowes Center employs one Illumina HiSeq 2000, three Illumina HiSeq 2500s, one Illumina MiSeq, and one ABI3730xl sequencer for next-generation sequencing applications, such as RNA-seq, chromatin immunoprecipitation with high-throughput sequencing (ChIP-seq), and assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq). The core will soon be adding the second-generation Pacific Biosciences sequencer (the Sequel), allowing extremely long sequencing reads (10-20 kB) at the genome level to probe structural variants, translocation events, and repeat disorders.

***Research Computing Center*.** The Research Computing Center provides high-performance computing resources, including an MPI cluster, a large memory server, a visualization server, GPU servers, and a high-performance storage pool to MCW researchers. Dr. Matt Flister, a Ph.D. computational chemist, manages the Research Computing Center network with another full-time staff member. Dedicated servers are installed to run ProteomeDiscoverer 2.4 (including Sequest HT, MS Amanda, Mascot search algorithms, Percolator, and ptmRS post-search validation tools), Byonic, ProSight PD, ProSight, Skyline with MS Stats, XCMS, Metlin, MetaboAnalyst, Agilent MassHunter, SPSS Statistics, ProteinCenter, Spectronaut, SPSS Statistics, Rosetta and AlphaFold (protein modeling), Desmond Molecular Dynamics Simulation software, and the Schrödinger Small-Molecule Discovery Suite.