

# EQUIPMENT

## Huck Institutes of the Life Sciences Shared Technology Facilities (University Park)

### Biomolecular Interactions:

Provides the necessary infrastructure and support for investigators to undertake studies of macromolecule binding and folding, as well as protein and lipid quantification. Specifically, we provide instrumentation and support for isothermal titration calorimetry (ITC) for studying ligand-macromolecule binding, differential scanning calorimetry (DSC) for investigating stability and folding thermodynamics, multiwavelength analytical ultracentrifuge (AUC) for determining the hydrodynamic shape, size, molar mass and binding thermodynamics of any macromolecule and its complexes, circular dichroism spectrometer (CD) to determine aspects of protein secondary and tertiary structure, and Lumicks C-Trap a single-molecule force microscope with two optical tweezers correlated with confocal-fluorescence and label-free microscopy. Our calorimetry instruments are fully automated and require the least amount of sample of any commercially available instruments.

#### *Instrumentation includes:*

Calorimetry instrumentation from a NSF-MRI award (DBI-0922974 to Bevilacqua, PI 2009); a Malvern isothermal titration system and a high throughput differential scanning calorimeter (AutoCAP-DSC). An automated TA Instruments Affinity ITC system (NIH 1S10OD025145-01, Yennawar, PI 2018). A Beckman Coulter multiwavelength Optima analytical ultracentrifuge (AUC) instrument equipped with both UV- visible absorbance and Rayleigh interference optics (1S10OD032215-01, Yennawar, PI 2022). A Lumicks C-Trap 2 allows for single molecule manipulation and analysis and force spectroscopy (NIH-1S10 OD038190-01 to Yennawar PI 2025)

### Cryo-Electron Microscopy:

Provides comprehensive support for Cryo-EM and Cryo-ET workflows, including—but not limited to—sample preparation, screening, data acquisition, and processing. Offers consultation for users initiating new projects as well as those facing challenges during their research. In addition, hands-on training is provided to ensure users are proficient in operating our advanced instrumentation.

#### *Instrumentation includes:*

A Thermo (FEI) Talos Arctica (NIH 1 S10 OD026822-01; Hafenstein et al, 2019) and a Titan Krios G3 are available. Typically, grid screenings are performed on the Arctica, and

datasets are collected on the Krios. The Krios is equipped with a Selectris X energy filter, Falcon 4i direct electron detector, Cs corrector, and Fringe-Free Imaging (FFI) capability, enabling efficient, high-quality, and high-throughput data acquisition. The Talos Arctica is also equipped with a Falcon 4i detector for high-throughput data collection. The humidity-controlled sample preparation suite is fully equipped with multiple vitrification systems, including a Vitrobot Mark IV, a Leica EM GP2 Freeze Plunger, an advanced Pin-Printing Technology VitroJet, as well as a Pelco Glow Discharge system for plasma cleaning of TEM grids. In addition, a Thermo Fisher Hydra Bio pFIB-SEM, a plasma-focused ion beam scanning electron microscope, is also available for cryogenic electron tomography (Cryo-ET) workflow.

### **CSL Behring Fermentation Facility:**

Provides state-of-the-art resources to support research, training and scale-up in microbial bioprocessing. Serves both academic and industrial users by offering scalable fermentation systems from bench-top to pilot scale for microbial cultivation, recombinant protein expression, metabolite synthesis, biomass production and high cell density cultures. Offers expertise in process development, optimization, downstream processing to help users design, test, and scale up bioprocesses efficiently while ensuring high-quality and reproducible outcomes.

#### *Instrumentation Includes:*

Bioreactor systems ranging from 500 mL to 500 L capacities to support small-to-pilot-scale microbial bioprocessing, including 7 x 500 mL Applikon MiniBioreactors, two BioStat B – Twin units (4 x 5 L), two BioStat Cplus units (2 x 30 L SIP), a BioStat D-DCU twin unit (1 x 50 L and 1 x 100 L SIP units), and a Sartorius SBC- 500L (500 L working volume) all with automation and data collection capabilities.

Microbial cell disruption and lysis is carried out using either a Microfluidics M-110EH or LM20 microfluidizers. Dialysis, buffer exchange, and concentration of products, such as recombinant proteins, is possible through Sartorius SARTOFLOW Smart and Advanced platforms for micro- and ultra-filtration. Chromatographic separation and purification of recombinant proteins, enzymes are performed using Cytiva AKTA pure 150 Fast Protein Liquid Chromatography. Additional support equipment such as incubated shakers, biosafety cabinets, and storage freezers ensures safe handling, preparation, and preservation of microbial cultures and bioproducts.

## **Flow Cytometry:**

Supports researchers with 24/7 access to advanced flow cytometry and performs BSL 2 enhanced (BSL 2+) flow sorting of samples derived from human, animals, plants, and bacteria for downstream applications or assays.

### *Instrumentation includes:*

Three flow analyzers (conventional and spectral modes) equipped with multi-lasers and -detectors. Cytex Aurora (4L and 5L UV/V/B/YG/R) instruments are widely used for deep immunophenotyping projects. In addition, the facility contains two flow sorter instruments, MACS Quant Tyto (V/B/R) microfluidics sorter for sorting fragile cells and Thermo Fisher Invitrogen Bigfoot (UV/V/B/YG/R) droplet sorter for high-speed bulk sorting of cells and particles for sub-population enrichment or selection; single-cell sorting for cloning and cell line derivation; and nuclei sorting for DNA sequencing. The facility provides training for students and researchers to use the instruments and supports designing of multi-chromatic cell-based and particle-based assays and high parametric flow cytometry data analysis using Kaluza and Flow Jo software.

## **Genomics:**

Offers next-generation library preparation and sequencing on both short and long read platforms. Sanger sequencing, fragment analysis, and whole plasmid sequencing are also offered. In addition, DNA and RNA sample QC analysis is supported.

### *Instrumentation includes:*

Illumina NextSeq 2000 and MiSeq, Pacific Biosciences Revio, ABI Hitachi 3730XL DNA Analyzer, Oxford Nanopore Technologies MinION Mk1C, Applied Biosystems QuantStudio 5 (2; one 96-well format and one in 384-well format), Sage BluePippin, Agilent Tape Station and Femto Pulse, ThermoFisher NanoDrop and Qubit, and three liquid handling robots (Agilent Bravo, Eppendorf epMotion 5075, and Hamilton MicroLab Prep).

## **High Field Magnetic Resonance Imaging:**

Offers pre-clinical magnetic resonance imaging and in vivo micro computed tomography. Besides ex vivo high-resolution MR microscopy and MR based material characterization, many state-of-the-art MR techniques can be applied in vivo on rodents, birds, fish, rabbits, and plants.

### *Instrumentation includes:*

A Bruker 14.1 tesla vertical microimaging system with a NEO console and two Tx/Rx channels for <sup>1</sup>H and one Tx/Rx channel for broadband imaging. A horizontal 7 Tesla Bruker 70/30 BioSpec with an Avance III HD console running Paravision 7.0 and 6.01. This system offers one Tx channel and four Rx channels for <sup>1</sup>H and one Tx and one Rx channel for broadband imaging. The Facility also houses a Bruker SkyScan 1276 in vivo micro CT.

## **Metabolomics:**

Offers nominal and accurate mass spectrometry of synthetic compounds and biomolecules. Our main goal is to identify and quantify small molecules (<1kDa) present in a wide variety of sample types including animal biofluids, cells, and tissues, bacteria, fungi, food products, water, soil, and plants. Comparing metabolites across samples can offer insights into the underlying biological processes involved in their biosynthesis. The facility's services include sample preparation, identification and quantitation by liquid chromatography-mass spectrometry (LC-MS, LC-MS/MS, LC-MSn) or gas chromatography-mass spectrometry as well as data analysis.

### *Instrumentation includes:*

Sciex QTRAP 6500+, Thermo Orbitrap Exploris 120, Thermo Orbitrap Exploris 240, Thermo TSQ Quantis Plus, Sciex ZenoTOF 7600, Agilent 5975 GC-MS, Thermo Orbitrap Astral, and Thermo Orbitrap Fusion Lumos (NIH 1 S10 OD021750-01A1; Patterson et al, 2017). The Metabolomics Facility also houses a Bruker NEO 600 NMR spectrometer with Bruker SampleJet autosampler (capacity >500 samples), for high throughput analysis. Spatial Metabolomics is made possible by the Bruker timsTOF flex MALDI-2 with resolution down to 5µm.

## **Microscopy:**

Provides conventional transmission- and scanning electron microscopy, cryo-SEM, cryo-TEM, digitized imaging, microtomy, cryogenic sample preparation, high resolution imaging, elemental analysis, immunogold labeling, freeze-substitution, histology and immunohistochemistry.

### *Instrumentation includes:*

1. Electron microscope: a Zeiss SIGMA VP-FESEM with Gatan cryo-stage, Oxford EDS, and Gatan 3View2 system.
2. Optical microscopes: a Zeiss LSM880 high-end confocal microscope with Airyscan Fast and PicoQuant FLIM, a Zeiss Lightsheet 7 Light Sheet microscope, a Leica SP8 DIVE multi-photon microscope, a Keyence BZ-9000 and an Olympus BX61 fluorescence microscope, a

Zeiss AxioZoom v16 fluorescent stereo zoom, and two super-resolution microscopes (Nikon N-SIM/N-STORM and BioVision VT-iSIM).

3. Equipment for histology preparation: a Leica TP1020 paraffin processor, a Leica EG1150G embedding unit, two Shandon Finesse paraffin microtomes, a Leica Autoslides stainer XL, and a Leica CM1950 Cryostat.
4. Other auxiliary equipment: Zeiss PALM Microbeam laser capture microdissection system, two Leica UC6 ultramicrotomes with cryo-unit, a Leica HPM100 high pressure freezer, and equipment for freeze-substitution and IHC, and a Leica CPD300 critical point dryer.

## **Proteomics:**

Provides mass-spectrometry-based analytical services for proteomics, chemical synthesis product, polymer, and biomolecule characterization.

### *Instrumentation includes:*

Thermo Q Exactive Orbitrap mass spectrometer with heated electrospray ionization probe or atmospheric pressure chemical ionization probe, interfaced with a Thermo Vanquish Flex liquid chromatography system: intact protein MW, small molecule qualitative and quantitative analyses, LC-MS and MS2 with reverse-phase, HILIC, and size-exclusion chromatography. Thermo Orbitrap Eclipse mass spectrometer capable of CID, HCD, EThcD, ETciD, and UVPD and MSn; supports TMT Pro in MS2 and SPS MS3 workflows; interfaced with a Thermo Vanquish Neo nano- and micro-flow liquid chromatography system: proteomics including post-translational modification analyses, pull-downs and other subproteome analyses, crosslinking and proximity labeling analyses of protein-protein interactions. The Core Facility users have access to the Thermo Proteome Discoverer 2.5 and Bioinformatics Solutions PEAKS software. Bruker Ultraflex extreme MALDI TOF-TOF mass spectrometer: peptides, proteins, and other biomolecules MS and MS2, polymer MW and other parameters with PolyTools software, library-based microorganism identification, Bruker MALDI Biotyper (library contains >8000 entries).

## **Sartorius Cell Culture:**

Supports research by providing mammalian cell cultures, media, supplies, and expertise on a recharge basis. The facility performs on site experimentation for researchers as well as providing cell cultures, supernatants, and cell pellets to researchers for experimentation in their own labs. Allows for onsite use of laminar flow hoods, incubators, bioreactors, and other equipment. Provides training in cell culture methods to students, staff, and faculty.

*Instrumentation includes:*

Sartorius AMBR 250 Modular bioreactor system, Sartorius Biostat BDCU controller for use with conventional stirred tanks (2L and 10 L) or single use tanks (2L) as well with the Biostat B Wave-mixed RM (rocker motion) Rocker with Flexsafe RM bags, Biostat STR 50 L and 250 L bioreactors, an Incucyte Live Cell Analysis System, Varioscan multimode plate reader, Sartorius Ultracentrifuge, 1<sup>o</sup>/minute cell freezer, and LN2 cell storage/recovery.

## **X-ray Crystallography:**

Provides the necessary infrastructure and support for individual investigators to undertake single crystal X-ray structural studies.

*Instrumentation includes:*

An NIH funded X-ray system (1S10RR023439-01, Yennawar, PI 2007, 1S10OD028589-01, Yennawar, PI 2020) including a Rigaku Micromax 007 X-ray generator equipped with a modern HyPix-Arc 150° Curved HPC Detector with a Universal Kappa 4-Axis Goniometer at the right port for single crystal X-ray diffraction data and a BioSAXS-2000Nano for small angle X-ray Scattering studies on the left port. The right port is configured with a VariMax-VHF high flux optics and Oxford Cryosystems COBRA for cryo-cooling the crystals. The BioSAXS port has a variable temperature, an OptiSAXS Confocal Max-Flux (CMF) SAXS Optic and an Automatic Sample Changer for high-throughput SAXS experiments. The core facility also houses a Wyatt Nanostar Dynamic Light Scattering instrument, an Art Robbins Instruments Phoenix protein crystallization robot, Formulatrix Rock Imager 182 with brightfield and UV microscope, Jasco J-1500 high performance Circular Dichroism spectrometer, Nikon SMZ18 stereo microscope with digital camera and a Jansi UV microscope. In addition, the facility houses state-of-the-art protein characterization equipment, an inline Wyatt size exclusion chromatography and inline multi-angle light scattering and refractive index system (1S10OD030490-01, Yennawar, PI 2021).