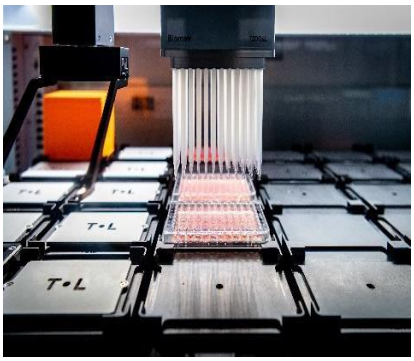
The Warren Roundup

Warren Family Research Center for Drug Discovery

Director's Note~

As the 2019 academic year comes to an end, a summary of this year's accomplishments is provided in the annual Warren Roundup. First of all, Notre Dame researchers have sought to develop drugs for the treatment of various diseases and several of these are undergoing preclinical or clinical evaluation. As can be seen in the articles below, several biotech/therapeutic companies have been started by Notre Dame Researchers, and in some cases, collaboration with the pharmaceutical industry has also taken place to further accelerate the development of novel drugs. In addition, new instrumentation has been acquired via grants and financial support from the Scholl's Foundation, which can be used by any member of the Warren Center. Pictures and details are provided on the last page of this newsletter. All three cores (computational, synthesis, and biological) are available to researchers at Notre Dame. Finally, the Warren Center recently received an endowment from the Leahy-Filipi Family to support a graduate student fellowship in the area of neuroscience research, with a focus on drug discovery. This fellowship in neuroscience complements a recently announced graduate fellowship in Cystic Fibrosis research by the Welter Family Foundation. A new RFA for each of these fellowships will be announced in the coming year.

Drugs in the Pipeline



Professors Mobashery and Chang continue to develop a drug for the treatment of diabetic foot ulcers, with the goal of starting clinical trials in the fall of 2021. Koren and Blagg are working with a venture capitalist firm and pharmaceutical company to develop a new treatment for glaucoma, which is hoped to enter clinical evaluation within the next 1–2 years. Hsiri Therapeutics (Miller and coworkers) signed a licensing agreement with Shionogi and hope to develop a new treatment for Non Tuberculosis Mycobacterial (NTM) infections and targeted anti-TB agents through this collaboration. Mishra and Blagg have spun off Grannus Therapeutics, which aims to develop new anti-cancer agents. Structured Immunity (Baker and colleagues) was launched in 2017 with the goal of partnering with established immunotherapy companies to help improve and optimize therapeutic pipelines via structural and computational biology. Having had initial success, the company is currently pivoting, with the aim of using its technology to develop immunological therapeutics for rare cancers. An investigational new drug, developed by the Blagg Lab, continues clinical evaluation for the treatment of neuropathy.

By the Numbers

Warren Center Researchers include 34 Principal Investigators, 44 Postdoctoral

Researchers, 126 Graduate Researchers, 17 Research faculty, and 63 Undergraduate Researchers. Combined, we published 127 scientific articles in 2019, 33 of which resulted from collaboration with other Warren Center scientists, whereas 48 resulted from interdepartmental collaborations across 16 departments on the Notre Dame campus. Warren researchers filed 37 US patents in 2019, whereas 67 US patents were issued and 14 start-up companies have been spun off to accelerate the drug discovery process. In addition, Warren researchers have requested more than $64,000,000 million in federal grant support through 66 proposals, 29 of which were awarded.

Celebrate Good Times

The second annual Warren Center Christmas Party was held in McCourtney Hall on December 14th to celebrate recent successes and to promote additional interactions. All PI's and their group members were invited to the "Winter Wonderland".

Approximately 170 people attended. Entertainment was provided by The Standard Deviants (band members include Professors Brian Baker and Shaun Lee). Great fun was had by all, and we look forward to celebrating new accomplishments next December.

Leahy–Filipi Graduate Fellowship

Francisco Huizar, a graduate student in the Zartman laboratory is the first recipient of the Leahy–Filipi Family Graduate Fellowship in Science for research in Neuroscience. The Leahy–Filipi graduate endowment was established in 2019 by Rev. Jody Leahy Filipi and Dr. Dave H. Filipi. David was diagnosed with a rare neurological disease, and together, they set up an endowment to help improve the life of others who also struggle with neurodegenerative diseases. With an astounding five million people in the United States suffering from Alzheimer's disease, and a projected 12 million people suffering from neurodegenerative diseases within the next 30 years, there is an urgent need to develop drugs that may significantly improve cognitive defects and neurodegeneration. The long-term goal of Huizar's project is to discover safe, more effective chemical inhibitors of DYRK1a, a protein linked to neurodegeneration and cognitive defects, to ameliorate Alzheimer's disease. The project is in collaboration with the Ashfeld group and has received seed funding from an AD&T Discovery award. The generosity of the Leahy-Filipi Family Endowment serves to provide a tremendous boost to his motivation as he strives to honor the efforts of Jody Leahy Filipi and Dr. David Filipi to improve the quality of life for those suffering from neurodegenerative diseases. Congratulations!

Three Researchers Awarded Grants to expedite Drug Discovery

The Warren Center for Drug Discovery and Development is a state-of-the-art resource for drug discovery researchers who have an interest in the development of molecular probes, drugs, chemical tools, biological screens or metabolomic assessments to study neurological and central nervous systems disorders, infectious disease, cancer, rare diseases or other issues of human health.

An RFA had been established to expand its interactions and collaborations on campus by providing resources within the core to support new and existing collaboration that utilizes the core's strength and expertise. Each researcher was awarded funds/services to cover the cost of research performed within the Warren Center.

Funding has been awarded to:

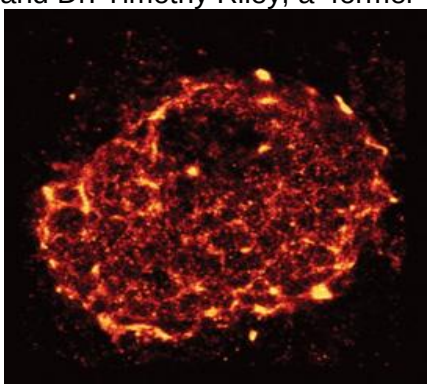
- **Geoffrey Siwo** from the Eck Institute for Global Health to work with Olaf Wiest in the CAMD Facility.
- **Brad Smith** from Chemistry and Biochemistry to work with Brandon Ashfeld in the CSDD Facility.
- **Xin Lu** from Biological Sciences to work with John Koren in the BSD Facility.

Congratulations!

Another request for applications will be announced during the fall of 2020 and will encourage utilization of the three cores.

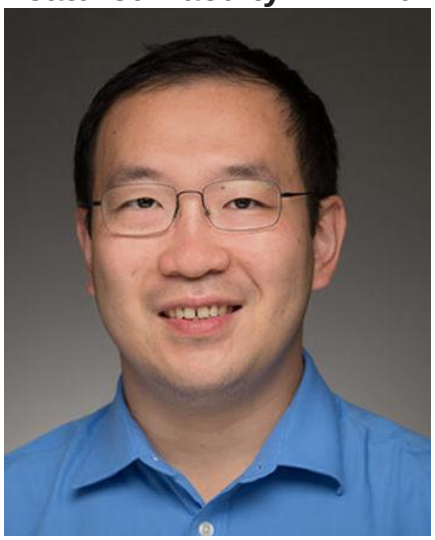
Structured Immunity

Structured Immunity began in 2017 as a Notre Dame biotech startup, led by Professor Brian Baker, Chair of the Department of Chemistry and Biochemistry, and Dr. Timothy Riley, a former



graduate student. The company's primary goal is to improve the potency and to reduce the side effects of T cell-based immunotherapies. T cells are a type of white blood cells that sense the presence of and eliminate diseased cells, such as those that are virally infected or cancerous. Their major focus is on accelerating and de-risking therapeutics that are based on the T cell receptor, the primary sensing molecule of T cells. Structured Immunity has expertise in structural biology, computational biochemistry, and immunology. When analyzing TCRs, Structured Immunity focuses first on the three dimensional structure of the receptor in complex with its target. As Professor Baker explained, "The availability of the structure allows the protein to be engineered in a manner that improves specificity, reducing the likelihood of off-target toxicity, which has been a considerable challenge for therapies based on these molecules." Additionally, according to Dr. Riley, "the structural information allows them to build hypotheses, test predictions, and improve the process." Recently, the company has been exploring moving into T cell based peptide vaccines. Structured Immunity can conduct the following assays: biochemistry, structural biology, blood cell assays, and cell-mediated immune responses. They also have efforts in discovery and preclinical analysis work for cell and molecular immunotherapy. They are actively forming partnerships with biopharmaceutical companies to help improve validation and optimization of TCR proteins for safer, more effective therapeutic uses. They partnered with Medigene AG, a leader in the development of immunotherapies for treatment of cancer. Structured Immunity will provide structural immunology expertise to support Medigene's TCR discovery activities. This collaboration combines the expertise of the research lab of Professor Baker and support from Notre Dame's IDEA Center.

Featured Faculty - Xin Lu



Xin Lu is the John M. and Mary Jo Boler Assistant Professor of Biological Sciences at the University of Notre Dame, the junior chair for the Boler–Parseghian Center for Rare and Neglected Diseases (CRND), and an associate member of the Indiana University Simon Cancer Center. He received a Bachelor's degree in Biology from Tsinghua University and a PhD in Molecular Biology from Princeton University (mentor: Yibin Kang). His postdoctoral research was conducted with Dr. Ronald DePinho, a former president of the University of Texas MD Anderson Cancer Center. Since beginning his career at Notre Dame in 2017, Dr. Lu's group has published more than 10 peer-reviewed manuscripts in leading journals such as *PNAS* and *Nature Communications.* Dr.

Lu's research at Notre Dame is supported by the NIH/NCI, Department of Defense, Susan G. Komen Foundation, Elsa U. Pardee Foundation, Mary Kay Foundation, Indiana CTSI, HCRI Walther Cancer Foundation and the Warren Center. Dr. Lu's laboratory is focused on the identification of cancer cell intrinsic and extrinsic mechanisms of tumor escape from immunosurveillance, particularly in metastatic prostate cancer and breast cancer. His recent publications firmly establish that immunosuppressive myeloid cells, especially those of the granulocytic lineage, play a predominant role at inducing the exhaustion of cytotoxic T lymphocytes in the prostate tumor microenvironment. His laboratory also investigates immunotherapeutic agents that target newly identified pathways in prostate cancer, breast cancer and rare cancers (penile squamous cell carcinoma, Von Hippel-Lindau syndrome). In essence, Dr. Lu is the "combination immunotherapy guy" on campus and welcomes new opportunities for collaboration.

Welter Family Graduate Fellowship in Science

Veronica Hubble, a graduate student in the Melander lab is the first recipient of the Welter Family (Terri and Mark Welter) Graduate Fellowship in Science for research on Cystic Fibrosis.
The Welter Family Fellowship in Science provides financial support to conduct research during the 2019-2020 academic year. This fellowship is focused on facilitating discoveries in cystic fibrosis research, which can include mechanistic studies, drug development, and/or related diseases, that affect those with CF (eg. infections of the lung). Veronica's proposal was entitled "Using small molecule adjuvants to combat antibiotic resistant bacteria in cystic fibrosis." Congratulations!

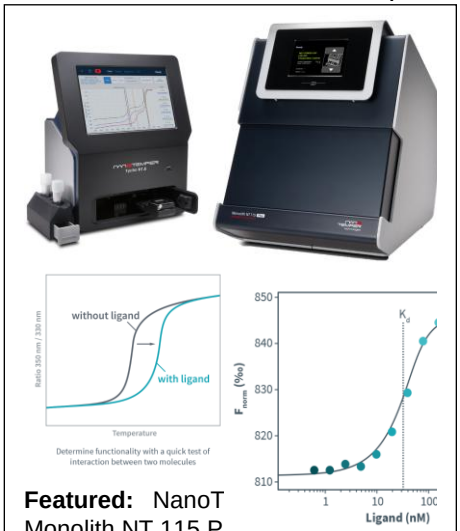
Molecular Targeting Technologies, Inc.

Molecular Targeting Technologies, Inc. (MTTI) a biotech company out of West Chester, PA has been established to advance novel medical imaging products for the diagnosis of cardiovascular disease and cancer. MTTI has also developed fluorescent probes and other research tools for use within the research community. Some of their current research projects include CellVue®, NeuroVue®, SRfluor®, (novel proprietary fluorescent squaraine-rotaxane dyes with excellent fluorescence properties) and novel immobilized steroid beads. In 2010, MTTI obtained an exclusive license from the University of Notre Dame for technology developed by Professor Bradley Smith, Ph.D., Emil T. Hofman professor of chemistry and biochemistry and Director of the Notre Dame Integrated Imaging Facility. Professor Smith designed a cell-sensing technology which can selectively target dead and dying mammalian cells as well as bacteria. This technology can target mammary and prostate tumors when the target compound is attached to a fluorescent probe. This led to study the target anionic cell surfaces to pursue molecular imaging of microbial infection which at the time was an area in need of more research. Due to this research, Professor Smith's lab was able to develop optical probes for photodynamic therapy of bacterial infection. They established that a set of molecular ZnDPA probes can selectively target parasite infections in living subjects. His lab developed an array of new fluorescent molecular probes, of which more than a dozen are commercially available through MTTI and can be used by researchers around the world. They are hoping to invent clinical imaging methods to improve patient care by expeditiously assessing effectiveness of cancer treatment or the extent of cardiovascular disease. According to Professor Smith, "this unique probe has the potential to image cell death as a means to intervene early in disease and rapidly determine the effectiveness of treatments." He also states that "Imaging cell death is broadly useful for treatment of numerous conditions, including

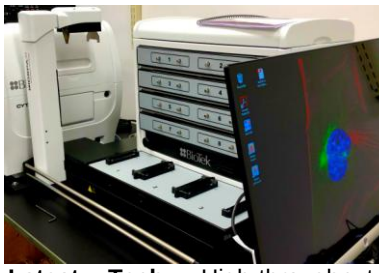
cardiovascular diseases, neurology, renal disease and even transplant rejection." This can be used for in vitro applications and in vivo imagining, "clinical success is not guaranteed but the journey will be fascinating," according to Professor Smith. His journey has led to new technologies that will eventually benefit many.

The Biological Screening & Development Core

The BSD core provides expertise and technologies to assist with and perform a range of assay and development services for drug discovery. These services include assay development and automation. We offer more advanced services including pharmacokinetic (PK), pharmacodynamic (PD), and bio-distribution studies for lead compound development using our Agilent Triple Quad LCMS – the gold standard for IND-enabling studies. We offer a full range of basic ADME-T services for small molecule development using high-throughput pre-clinical screenings; these assays include: Microsomal Stability in human liver microsomes, Primary Hepatocyte Toxicity, Caco-2 Permeability and Transport, Human Plasma Stability, Human Plasma Protein Binding, hERG Interaction, AMES Mutagenicity Test for frameshift and point mutations, and Blood-Brain Barrier Permeability (via PAMPA). The core is capable of generating and purifying recombinant proteins for numerous in vitro assays. Our newest technologies include devices for Kd determinations (ligand-protein or protein-protein) using our new Nanotemper Microscale Thermophoresis platform and an automated platform for live cell imaging. This live cell imaging automated platform, an system featuring an automated BioSpa cell culture incubator integrated with a Cytation5 microscopy system capable of imaging 96-well plates with three-channel fluorescence as well as bright field. This technology can be utilized on both 2D and 3D culture systems. The technology and devices are in place, however the assays require development. Information and protocol white pages are available from the BioTek website or can be sent to you at your request via Dr. Koren. We are currently looking for collaborations to help build these technologies. These technologies were added to our additional devices and systems including a state-of-the-art Biomek i7 Dual-Arm Liquid Handling Automation Platform, Cytation5 multimodal plate-reader with our ALPHA capabilities, tunable fluorescence and luminescence, our dedicated Agilent 6460 triple quad LC-MS with 1290 autosampler, and GE's AKTA FPLC to deliver reliable data in a timely and cost-effective manner. Professor John Koren III is director of the AMDET/DMPK facility and runs the facility with his staff scientist Monimoy Banerjee and technician Vitumbiku Munthali. For more information, please contact:



Featured: NanoT Monolith NT.115 P... Tycho – Capillary based label-free detector of protein purity or ligand-protein interactions.
Monolith – Quantitative determination of Kd values from protein-protein or ligand-protein interactions by MicroScale thermophoresis.



Latest Tech: High-throughput Imaging - BioTek Cytation 5 microplate Imaging system with integrated automated BioSpa cell culture incubator. System has three channel fluorescent filters and brightfield capabilities. System has 4x, 10x, and 20x

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The Chemical Synthesis Core

The Chemical Synthesis and Drug Discovery (CSDD) Facility that is overseen by Professor Brandon Ashfeld supports translational biomedical research by providing expertise that enables the preparation of small molecules for use in hit verification, lead development, and midsize scale up. In addition, the core prepares biological probes (affinity or fluorescently tagged), active pharmaceutical agents as

experimental controls, and small chemical libraries for structure-activity relationships as well as the optimization of pharmacological properties. The CSDD coordinates the organizational oversight of compounds from past, current, and future chemical synthesis endeavors that comprise the Notre Dame Chemical Compound Collection, which currently contains ~5000 unique chemical entities. The CSDD is staffed with PhD level scientists (research scientists and postdoctoral research associates) with expertise in multi-step organic synthesis, medicinal chemistry, parallel development, purification and isolation of small molecules. Services that are offered include, but are not limited to:

- Parallel synthesis of small molecule libraries
- Single compound preparation (10 mg to 20 g)
- Synthesis of biological probe molecules
- Peptides Synthesis
- Purification of complex mixtures
- Sample plating and distribution
- Project development with a special emphasis on therapeutics development
- Consultation

For more information, please contact:

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The Computational Core

The Notre Dame Computer Aided Molecular Design (CAMD) Core Facility of the Warren Family Research Center for Drug Discovery and Development provides the full range of computational chemistry support, from atomistic modeling to assistance in proposal writing for drug discovery and related areas to all groups on campus. CAMD computational scientists have extensive expertise in virtual screening for inhibitor design, including docking, scoring, MM/PBSA calculations, library design and cheminformatics. CAMD expertise in molecular dynamics extends from standard MD to advanced methods, such as Long Timestep Molecular Dynamics (LTMD), to Free Energy Perturbation and Nudged Elastic Band simulations. In the area of electronic structure calculations, the CAMD uses density functional theory (DFT), correlated quantum mechanics (QM) and hybrid quantum/classical calculations (QM/MM) methods. In addition to utilizing existing methodology, CAMD is actively developing new methods, such as Q2MM and Ensemble Rescoring. CAMD expertise extends to pharmacokinetics and predictive modeling, encompassing quantitative structure-activity relationships (QSAR), cheminformatics, library and ligand-based design, and network analysis. For more information, please contact:

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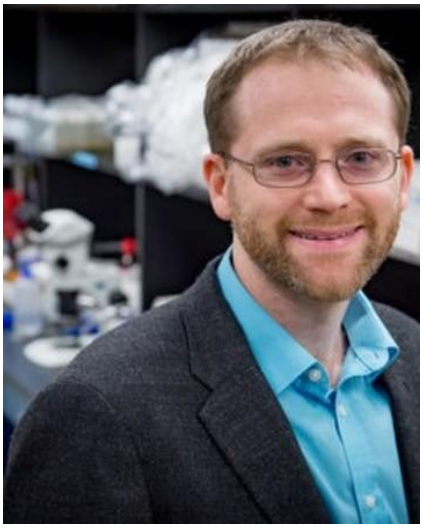
Featured Faculty – Jeremiah Zartman



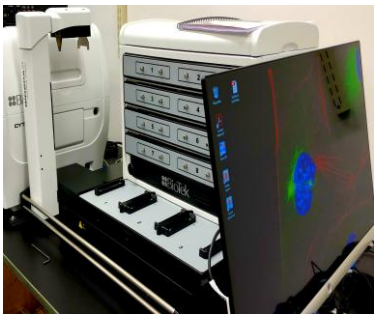
Professor Jeremiah Zartman is an Associate Professor with tenure in the Department of Chemical and Biomolecular Engineering and Bioengineering Ph.D. program. Jeremiah received his B.S. degree from the University of Colorado at Boulder with a dual major in Chemical Engineering and Engineering Physics in 2004. In 2009 he obtained his Ph.D. in Chemical and Biomolecular Engineering under the supervision of Prof. Stanislav Shvartsman at Princeton University as a Princeton Hertz Fellow. From 2009-2011, he worked as a post-doctoral scientist in the lab of Prof. Konrad Basler, University of Zurich, in Molecular Life Sciences as an EMBO Long-term Post-doctoral Fellow. Since 2012, Dr. Zartman has led a research group at the University of Notre Dame. Dr. Zartman also joined the Editorial Board of Biophysical Journal in 2020. His research group, based in McCourtney Hall, focuses on the integration of computational and experimental approaches to reverse engineer the development and function of multicellular systems from a systems perspective. His group also develops advanced in vivo

screening approaches for target discovery and preclinical testing of new therapeutics with applications including cancer, neurodegeneration and regenerative medicine. Dr. Zartman received the NSF CAREER award in 2016, the 2018 Biomedical Engineering Society “Rising Star” award, and the 2019 class of Michiana Forty under 40.

New Instruments for use by Warren Center

Cytation 5 Integrated with a BioSpa Cell Culture Incubator.
Located immediately adjacent to our Beckman i7 Dual-Armed automation platform, these systems dramatically enhance Notre Dame’s drug screening and assay development capabilities.

- Proliferation assays
- Automated Wound healing/ Scratch assays
- 2D and 3 D cultures
- Cancer cell line, primary cell lines and stem cell lines
- On-demand scheduling
- Throughput workflow



Cytation™ 3 Cell Imaging Multi- Mode Reader
Three-dimensional (3D) cell culture has become a well-established in vitro experimental approach as it provides an improved in vivo-like environment. The use of clear U bottom ultra-low attachment microplates that minimize cell adherence has become a standard for applications such as spheroid proliferation. Results shown here demonstrate the ability to generate quality results using both brightfield and fluorescence microscopy.

- Uses 3D Cell Culture
- Spheroid
- Hanging Drop Fluorescence
- Imaging Liver Microtissue
- Hypoxia

Cytation 5 Cell Imaging Multi-Mode Reader
Cytation 5 is a uniquely integrated, configurable system that combines automated digital widefield microscopy with conventional multi-mode microplate detection to provide phenotypic cellular information and well-based quantitative data.

- Microscope objectives(4x, 10x, & 20x
- Capable of brightfield(with and without phase contrast

Three channel fluorescent imaging (DAPI, RFP, & GFP)

Warren Lecture Series/ Seminar Speakers

The Warren Center has established the Warren Lecture Series to invite guest lecturers to inform, share discoveries and scientific expertise within the Warren community. In 2019 Warren Center sponsored six guest lectures. You can nominate a guest lecturer with strong expertise in drug discovery via email to wrcadmin@nd.edu or website at https://drugdiscovery.nd.edu/ seminars.