

## Professor Alexander (Leggy) Arnold from Milwaukee Institute for Drug Discovery



We interviewed Dr. Arnold, aka Leggy Arnold, who is an Associate Professor in the Department of Chemistry and Biochemistry, Principle Investigator of the Shimadzu Laboratory for Advanced and Applied Analytical Chemistry and Founding Member of the Milwaukee Institute for Drug Discovery (MIDD) at the University of Wisconsin-Milwaukee (UWM). One area of his research is focused to elucidate the biological and pharmacological role of vitamin D receptor–coregulator interaction by using small molecule probes that in turn are developed into drug candidates for metabolic disease and cancer. His lab supports high throughput screening, medicinal chemistry, biochemistry and molecular biology. The relationship with Shimadzu started in 2014, when the “Shimadzu Laboratory for Advanced and Applied Analytical Chemistry” was established within the MIDD.

**Dr. Arnold, thank you very much for spending some time for this interview. At first, could you outline the research and let us know what discovery and achievements have been made so far?**

Our research is focused on the identification of new synthetic and natural compounds that modulate the function of the vitamin D receptor. The vitamin D receptor is a transcription factor that induces the production of RNA molecules, which in turn are used to generate essential proteins in our body. The vitamin D receptor, as the name implies, binds vitamin D and its metabolites. We have been working together with the NIH Chemical Genomics Center and applied high throughput screening to find new compounds that modulate the function of the vitamin D receptor. We have developed several different compounds that bind the receptor directly or those that bind the receptor surface to modulate the interaction with coregulatory proteins that are essential for gene transcription. We observed that a specific irreversible inhibitor class of compounds exhibited antiproliferative effects. Based on that finding, we developed a compound against leukemia and another compound that reduces the growth of ovarian tumors.

**Why are you interested in this research? What is the goal?**

We are all familiar with vitamin D as an essential vitamin that prevents bone diseases, improves kidney function and is an important molecule for healthy skin and hair growth. The molecule that regulates the majority of processes is the vitamin D receptor. I believe that we can target the vitamin D receptor with small molecules to fight diseases that need this receptor in order to thrive. One of the most used chemotherapeutics in the United States for fighting breast cancer is Tamoxifen. Tamoxifen is binding a closely related receptor called the estrogen receptor. Like the vitamin D receptor, the estrogen receptor is essential for many life processes. We are able to target this receptor with small molecules to successfully fight breast cancer. I'm convinced that the vitamin D receptor has a similar potential and can be targeted by smart compounds to reduce the growth of tumors especially those that express high levels of this receptor like skin cells, white blood cells, and many more. In addition, we continue our work on the development of new treatments for kidney diseases, osteoporosis and immune diseases.

**Could you tell us why you chose Shimadzu as your partner when you established this new lab?**

In particular, our research is supported by the MIDD. Our director, Dr. Douglas Stafford, worked in conjunction with Shimadzu to establish an “Analytical Chemistry Center of Excellence” at the UWM. Dr. Stafford was able to secure funding from the University of Wisconsin System for a Center concept to not only support basic research across our campus, but also serve as a regional resource for industrial collaborations and chemistry education. Shimadzu was very generous by providing matching funds in form of instrumentation and long-term support and therefore enabled the establishment of one of the most sophisticated mass spectroscopy facilities in the nation.



**How are our instruments helping you?**

Our Shimadzu Laboratory for Advanced Applied and Analytical Chemistry facility is equipped with a range of new instruments and is housed in a brand new 2,000 sq. ft. laboratory in UWM's new Kenwood Interdisciplinary Research Center. Our instrumentation includes Shimadzu products such as the MALDI-7090 with AccuSpot and CHIP. We and other researchers are using this flagship MALDI TOF-TOF platform for proteomics. In particular, our group is identifying the binding site of our irreversible VDR inhibitors. In addition, we are conducting metabolomic research by identifying new catabolic products of vitamin D to determine their biological activity in respect to the vitamin D receptor. Our group is interested in pinpointing the most metabolically active tissues for the conversion of vitamin D to the most potent ligand of VDR 1,25-dihydroxyvitamin D<sub>3</sub>.

Our LCMS-IT-TOF is particularly useful for high resolution mass spectrometry. Several years back only a tri-sector mass spectrometer could achieve such resolution, however we routinely determine the exact mass of new compounds synthesized here at UWM and our neighboring institutions and universities. In addition, we are using the unlimited fragmentation capabilities of this instrument for structural analysis of unknown metabolites of vitamin D. Our vitamin D receptor–coregulator inhibitors have anti-tumor activities in respect of leukemia and ovarian cancer. We are utilizing our LCMS-8040 Triple Quad instrument with high speed positive/negative ionization switching for the quantification of drug candidate characteristics such as microsomal stability, pharmacokinetics, and tissue distribution. In addition to our specific drug discovery and development programs, instruments such as the GCMS QP-2010 Ultra with DI probe, LCMS-2020 single quad, FTIR-IR Tracer NIR/FAR, and a UV-2600 UV-Vis Spectrophotometer support the wide range of basic research within our Department of Chemistry and Biochemistry. The Shimadzu Laboratory is meeting its goal to be a unique platform for both research and chemistry education.

### What are Shimadzu's strengths compared to other vendors (not limited to the instruments)?

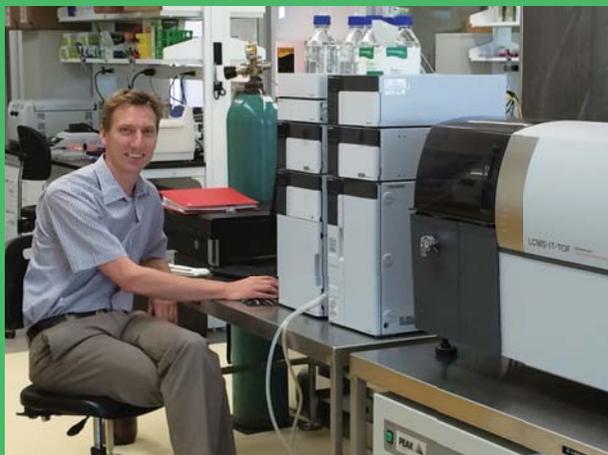
We are especially satisfied with the Shimadzu software. It's very intuitive and user friendly and has common operating design across the various instruments. It has several safety features that prevents operating errors and encourages preventative maintenance. This is especially important with a large user group, including a large number of students, and a multidisciplinary institute like the MIDD. More importantly, Shimadzu has excellent support with respect to people and knowledge. I would like to highlight one particular technical specialist Dr. Nishi Rochelle. As a talented analytical chemist, she has trained many of your staff and students and helped design and troubleshoot crucial first experiments that lead to preliminary results for our NIH and NSF grants and jumpstarted new collaboration and research. Many other excellent Shimadzu employees have given us special attention, which differentiates Shimadzu from other instrument companies and enabled the rapid start-up of a very sophisticated center.



### Finally, could you share any requests that you have with respect to analytical and measuring instrument vendors?

We are very happy with our new instrumentation platform, which is already having tremendous impact on cross-disciplinary research and education at UWM. Yet, the analytical facility is relative young and we are still exploring expanded areas of research as we look towards new collaborations with both academic and industrial partners.

**It was significant to know what you think of us and our collaboration. We will strive to meet your request more than ever. Thank you very much.**



#### Here are his recent publications:

- Margaret L. Guthrie, Preetpal S. Sidhu, Emily K. Hill, Timothy C. Horan, Premchendar Nandhikonda, Kelly Teske, Feleke, Nina Y. Yuan, Marina Sidorko, Revathi Kodali, James M. Cook, Lanlan Han, Nicholas R. Silvaggi, Daniel D. Bikle, Richard G. Moore, Rakesh K. Singh, Leggy A. Arnold "Anti-tumor activity of 3-indolylmethanamines 31B and PS121912" *Anticancer Research* **2015** accepted.
- Preetpal S. Sidhu, Kelly Teske, Belaynesh Feleke, Nina Y. Yuan, Margaret L. Guthrie, Grant B. Fernstrum, Nishita D. Vyas, Lanlan Han, Joshua Preston, Jonathan W. Bogart, Nicholas R. Silvaggi, James M. Cook, Rakesh K. Singh, Daniel D. Bikle, Leggy A. Arnold "Anticancer Activity of VDR-Coregulator Inhibitor PS121912" *Cancer Chemotherapy and Pharmacology* **2014**, 74(4), 787-798.
- Kelly Teske, Premchendar Nandhikonda, Jonathan W. Bogart, Belaynesh Feleke, Preetpal Sidhu, Nina Yuan, Joshua Preston, Robin Goy, Rakesh K. Singh, Daniel D. Bikle, James M. Cook, Leggy A. Arnold "Identification of VDR antagonists among nuclear receptor ligands using virtual screening" *Nuclear Receptor Research* **2014**, 1, 1-8.
- Preetpal S. Sidhu, Nicholas Nassif, Megan M. McCallum, Kelly Teske, Belaynesh Feleke, Nina Y. Yuan, Premchendar Nandhikonda, James M. Cook, Rakesh K. Singh, Daniel D. Bikle, Leggy A. Arnold "Vitamin D Receptor-Coactivator Binding Inhibitors" *ACS Med Chem Lett* **2014**, 5(2), 199-204.
- Kelly Teske, Premchendar Nandhikonda, Jonathan W. Bogart, Belaynesh Feleke, Preetpal Sidhu, Nina Yuan, Joshua Preston, Robin Goy, Leggy A. Arnold "Modulation of Transcription mediated by the Vitamin D Receptor and the Peroxisome Proliferator-Activated Receptor  $\delta$  in the presence of GW0742 analogs" *Journal of Biomolecular Research and Therapeutic* **2014**, 3, e111.
- Megan M. McCallum, Alan J. Pawlak, William R. Shadrack, Anton Simeonov, Ajit Jadhav, Adam Yasgar, David J. Maloney, Leggy A. Arnold "A Fluorescence-Based High Throughput Assay for the Determination of Small Molecule-Human Serum Albumin Protein Binding" *Analytical and Bioanalytical Chemistry* **2014**, 406(7), 1867-1875.
- Katherine M.J. McMurray, Margaret G. Distler, Preetpal Sidhu, James M. Cook, Leggy A. Arnold, Abraham A. Palmer, Leigh D. Plant "Glo1 inhibitors for neuropsychiatric and anti-epileptic drug development" *Biochemical Society Transactions* **2014**, 42(2), 461-467.
- Nada Kawar, Shannon Maclaughlan, Timothy C. Horan, Alper Uzun, Thilo S. Lange, Kyu K. Kim, Russell Hopson, Ajay P. Singh, Preetpal S. Sidhu, Kyle A. Glass, Sunil Shaw, James F. Padbury, Nicholi Vorsa, Leggy A. Arnold, Richard G. Moore, Laurent Brard, and Rakesh K. Singh "PT19c, Another Nonhypercalcemic Vitamin D2 Derivative, Demonstrates Antitumor Efficacy in Epithelial Ovarian and Endometrial Cancer Models" *Genes and Cancer* **2013**, 4(11-12), 524-534.
- Premchendar Nandhikonda, Adam Yasgar, Athena M. Baranowski, Preetpal S. Sidhu, Megan M. McCallum, Alan J. Pawlak, Kelly Teske, Belaynesh Feleke, Nina Y. Yuan, Chinedum Kevin, Daniel D. Bikle, Steven D. Ayers, Paul Webb, Ganesha Rai, Anton Simeonov, Ajit Jadhav, David Maloney, Leggy A. Arnold "PPAR $\delta$  agonist GW0742 interacts weakly with multiple nuclear receptors including the vitamin D receptor" *Biochemistry* **2013**, 52, 4193-4203.
- Megan M. McCallum, Premchendar Nandhikonda, Jonathan J. Temmer, Charles Eyermaun, Anton Simeonov, Ajit Jadhav, Adam Yasgar, David Maloney, Leggy A. Arnold "High-Throughput Identification of Promiscuous Inhibitors from Screening Libraries with the Use of a Thiol-Containing Fluorescent Probe" *Journal of Biomolecular Screening* **2013**, 18(6), 705-713.