Bryostatin, PKC, and HIV Latency Reversal

With practical application of our technologies at the forefront of our efforts, we are applying our exquisite NMR sensitivity to structural studies of protein kinase C (PKC) activation with bryostatin analogs, as applied to HIV cure research but also relevant to other diseases including cancer and cardiovascular disease. Although highly-active antiretroviral therapy (ART), a drug cocktail which prevents viral reproduction and integration into human cells, is very effective and reduces viremia below detectable levels, viral genetic reservoirs remain during treatment. This latent viral DNA resides within long-term CD4+ memory T cells and must be destroyed in order to truly cure, and eventually eradicate, HIV/AIDS.

We recently demonstrated that a synergistic combination of bryostatin analogs and isoform-targeted histone deacetylase inhibitors potently activate latent HIV without global T cell activation. This is a highly significant result in HIV cure research that aims to target the latent HIV reservoir for therapeutic benefit. This study was performed in close collaboration with George Kyei, WUSTL Medical School, acting as joint corresponding author on the study led by graduate student, Brice Albert. This paper, recently published in Scientific Reports, extends our work outside the specialist field of magnetic resonance and provides evidence of the extended reach, impact, and biomedical translatability of this science outside the immediate field of NMR and DNP. Demonstrating the utility of MAS-DNP on important biomedical targets is critical to attracting competitive funding, industrial collaboration, and inward investment.

We have devoted our laboratory’s application efforts to the structural characterization of bryostatin and PKC activation. PKC controls many disease-relevant cellular pathways, yet atomic resolution structural knowledge of PKC activators such as bryostatin is lacking. With this specific biomolecular target in mind, I have also focused on 19F-, 13C-, and 2H-labeled bryostatin to also...
achieve spectral resolution in low field (7 T) and cryogenically broadened spectra. Bio-orthogonal labels will also yield excellent signal above the background of intact cells as we move our SSNMR structural biology analyses into the cellular context.

In collaboration with Lynette Cegelski and Paul Wender at Stanford University and Jacob Schaefer at WUSTL, we have already characterized the structural ensemble of bryostatin bound to PKC. From these conventional NMR experiments (41 days per REDOR measurement, without DNP), we showed that bryostatin adopts multiple conformations, mirroring our molecular dynamics simulations.

**Connection:** The structural analysis of PKC activation with bryostatin and other ligands provides a robust and relevant biological exemplar for our magnetic resonance technology and methodology development. With even relatively modest improvements in NMR sensitivity, we will be able to shorten the month-long in vitro REDOR experiments to a matter of minutes for the study of PKC activation within membranes.

**Future Directions:** Along with our demonstration of in-cell DNP <6 K, we are now preparing to determine the structure of isotopically-labeled bryostatin in human cells harboring latent HIV. In addition to in-cell NMR, we are also currently performing MAS-DNP experiments on isotope-enriched PKC regulatory domains and activating ligands embedded in homogenous and heterogenous vesicles.