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Innovation in action:

University of Pittsburgh chooses Reichert SPR Systems twice

Thomas E. Smithgall, PhD uses Reichert SPR to research therapeutic inhibitors for treatments of cancer and infectious diseases.

Dr. Thomas Smithgall, Professor and Chair of the Microbiology and Molecular Genetics Department at the University of Pittsburgh, is performing research focused on characterizing new drug targets as potential therapeutic inhibitors to treat cancer and infectious diseases such as HIV. A target that has become of high interest to Smithgall's lab is HIV-1 Nef, a small membrane-associated protein that is critical for HIV-1 replication in vivo, immune escape of infected cells, and AIDS progression. Existing antiretroviral therapy does not remove the HIV virus from the body and requires life-long administration to prevent relapse. In addition, cumulative antiretroviral drug exposure could lead to clinical metabolic disturbances and organ damage. To address these issues, the Smithgall lab is focusing on the development of new classes of compounds, particularly small molecules that interfere with the functions of HIV-1.

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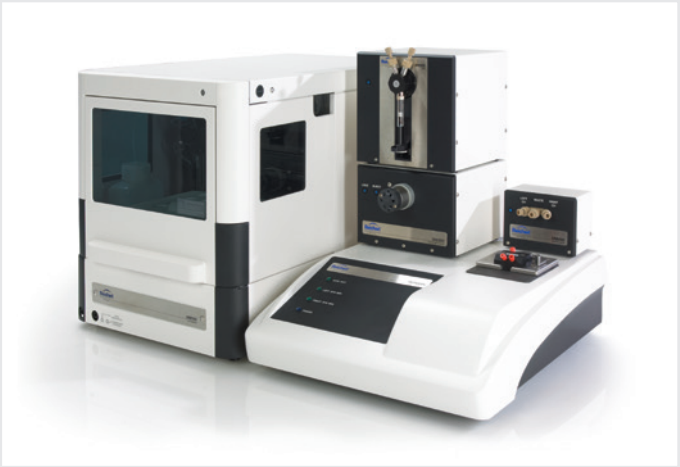
Need for Interaction Analysis

To advance this research, Smithgall evaluated potential techniques to monitor the binding of small molecule inhibitors to the Nef protein target. As Smithgall pointed out, "Nef has no enzymatic activity or biochemical readout and HIV-replication assays do not report on target binding" limiting the use of traditional end-point analysis methods for characterization. Smithgall turned to surface plasmon resonance (SPR) approximately four years ago to develop binding assays to characterize inhibitor-Nef interactions.

The instrument that was initially utilized was a GE Biacore 3000 instrument, followed by a GE Biacore T100. Both instruments were installed in a core facility at the University of Pittsburgh. As this HIV research progressed, Smithgall collaborated with two outside partners, focusing on medicinal chemistry to synthesize potential Nef inhibitors. As multiple potential Nef inhibitors were identified, the demand for SPR analysis grew and scheduling time on the Biacore instruments became a big bottleneck. So Smithgall applied for a grant and received funding through the NIH to procure an SPR system for his own laboratory.

Smithgall Lab Decides on Reichert 2-channel Reichert 2SPR System



The Smithgall lab evaluated several SPR platforms to identify a system that would not only accomplish their very demanding small molecule analysis applications, but would also meet budget requirements. After several on-site demonstrations, they found that Reichert Technologies and GE Biacore were the only vendors providing SPR systems with the essential sensitivity and performance. After comparing features and costs of a new GE Biacore system to the Reichert 2-channel Reichert 2SPR system, Smithgall decided on the Reichert system. He maintains that it was "no contest" Reichert was the obvious choice, compared to GE for an individual lab. Smithgall also pointed out that it was not just the lower cost of the Reichert system that factored into the decision. Smithgall said, "It was the overall package that Reichert offered in terms of having a system with outstanding sensitivity and significantly lower operating costs, and a very responsive top-notch customer service team with an expert knowledge of SPR that made the decision to purchase a Reichert SPR system extremely easy." In addition, Dr. John Jeff Alvarado, research assistant professor in the Smithgall lab, commented, "the open architecture design of the Reichert system was a very attractive feature in reducing potential instrument downtime." Alvarado also noted that Reichert's outstanding customer service and support also provided comfort in the decision remarking, "knowing that we would not have to get in line for service that could be a 2-week lead time before getting a response was also a key factor in the decision."

Smithgall Lab Decides on Reichert Again with a Reichert 4SPR System

As the number of small molecule inhibitors they needed to characterize significantly increased along with other interaction analysis work in their lab requiring SPR analysis, Smithgall realized the imminent need for further SPR capacity. He then applied for an NIH supplement grant to acquire a higher throughput SPR system. The Smithgall lab decided to stay with Reichert and purchase the Reichert 4SPR 4-channel system. As Smithgall states, "the decision to purchase the Reichert 4SPR system was very easy given the outstanding experience we had with Reichert and the 2-channel system." Furthermore, Smithgall commented, "The Reicher t4SPR system was the best combination of price and performance for our demanding small molecule application." The 4-channel system has been the cornerstone for advancing Smithgall's HIV research. "The ability to investigate multiple versions of proteins simultaneously is huge for us in reducing the time to obtain high quality results," said Smithgall.

Smithgall's Experience with Reichert

Currently, the Smithgall lab is using the Reichert 4SPR system around the clock. One of the main users of the system, Dr. Haibin Shi, research instructor for the Smithgall lab, commented, "the Reichert 4SPR system has provided a huge amounts of data. We run the system for 20 hours a day, 6 days a week." Most importantly, Shi points out they "analyzed a whole series of Nef analogs in a short period of time and produced very high quality results with confidence." Smithgall noted that his lab still uses the Reichert 2SPR for lower throughput experiments and for additional capacity when the 4-channel is in use. Smithgall emphasized there is no end in sight for the SPR experiments. His team is using docking models to identify additional inhibitors to Nef, which will need characterization with SPR. In addition, the Smithgall lab also plans on performing screening campaigns on allosteric inhibitors of protein kinases of the Src-family that pertain to cancer research. "The ability to determine the binding resonant time of small molecules to targets of interest is invaluable to us," Smithgall maintains. Furthermore, he added, "our Reichert SPR systems will continue to be an integral part of this research program for years to come."

Smithgall is Enormously Satisfied with the Decision

Smithgall summarized his experience thus far, "Reichert has been all-around terrific. The systems are very high quality and the support staff is very responsive to instrument and application questions making it very easy to work with them." Smithgall is extremely pleased with his decision to purchase the Reichert SPR systems and noted that they have been a "great investment" for his laboratory.

Reichert SPR Systems

- *Better sensitivity*
- *Broader range of experiments*
- *Higher throughput and uptime*
- *Self-maintained*
- *Lower capital and running costs*

Learn more about how Reichert pushes the limits of detection and sensitivity in label-free interaction on XanTec's website.

1. Badger, J., Grover, P., Shi, H., Panjarian, S. B., Engen, J. R., Smithgall, T. E., & Makowski, L. (2016). c-Abl tyrosine kinase adopts multiple active conformational states in solution. Biochemistry, 55(23), 3251-3260.
2. Emert-Sedlak, L. A., Loughran, H. M., Shi, H., Kulp, J. L., Shu, S. T., Zhao, J., ... & Smithgall, T. E. (2016). Synthesis and evaluation of orally active small molecule HIV-1 Nef antagonists. Bioorganic & medicinal chemistry letters, 26(5), 1480-1484.
3. Wales, T. E., Hochrein, J. M., Morgan, C. R., Emert-Sedlak, L. A., Smithgall, T. E., & Engen, J. R. (2015). Subtle dynamic changes accompany hck activation by HIV-1 Nef and are reversed by an antiretroviral kinase inhibitor. Biochemistry, 54(41), 6382-6391.
4. Tarafdar, S., Poe, J. A., & Smithgall, T. E. (2014). The accessory factor Nef links HIV-1 to Tec/Btk kinases in an Src homology 3 domain-dependent manner. Journal of Biological Chemistry, 289(22), 15718-15728.
5. Alvarado, J. J., Tarafdar, S., Yeh, J. I., & Smithgall, T. E. (2014). Interaction with the Src homology (SH3-SH2) region of the Src-family kinase Hck structures the HIV-1 Nef dimer for kinase activation and effector recruitment. Journal of Biological Chemistry, 289(41), 28539-28553.
6. Pirrone, G. F., Emert-Sedlak, L. A., Wales, T. E., Smithgall, T. E., Kent, M. S., & Engen, J. R. (2015). Membrane-associated conformation of HIV-1 Nef investigated with hydrogen exchange mass spectrometry at a Langmuir monolayer. Analytical chemistry, 87(14), 7030-7035.
7. Iyer, P. C., Zhao, J., Emert-Sedlak, L. A., Moore, K. K., Smithgall, T. E., & Day, B. W. (2014). Synthesis and structure–activity analysis of diphenylpyrazolodiazene inhibitors of the HIV-1 Nef virulence factor. Bioorganic & medicinal chemistry letters, 24(7), 1702-1706.

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