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Life Sciences

CASE STUDY
USING REICHERT SPR SYSTEMS

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Biochemist Mark Sutton, PhD uses Reichert SPR system to study weak molecular interactions involved in mutations and DNA repair

Mark Sutton, PhD, a professor of Biochemistry at the State University of New York at Buffalo, is using a Reichert surface plasmon resonance (SPR) instrument to understand the complex molecular interactions involved in the mechanism of DNA repair.

"My lab is interested in understanding the mechanisms of DNA replication, repair and damage tolerance," Sutton said in an interview with Reichert Life Sciences. "We're examining the role of polymerase enzymes by looking at bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* because they are prokaryotic organisms," he says.

Bacteria such as *E. coli* and *P. aeruginosa* have multiple polymerases compared to eukaryotic organisms that utilize 15 different polymerases. Because bacteria have a simpler DNA repair network, they have provided Sutton with a valuable model to examine the cell's molecular efforts in response to DNA damage and aid in replication. Sutton notes that "some of these polymerases are important for accurate replication of chromosomes, others can work on damaged or distorted templates, and others can cause mutations."

Examining Molecular Interactions

Sutton started his research 15 years ago by looking at protein-protein and protein-nucleic acid interactions that might determine how DNA polymerases were directed and the resulting effect. He examined the replication fork consisting of many replication enzymes including DNA polymerases.

To understand these interactions, he needed methods that would determine the strength, location, and kinetics of these interactions. Sutton tried gel filtration, isolation calorimetry and other methods, all proving very difficult in determining reliable kinetic information. "It was very challenging to deconvolute data when having multiple contacts between proteins," he stated. Using fluorescence also was problematic, because he had to tag proteins, which could affect the activity of that protein. "Just putting on a His-Tag affects function in vivo," he remarked.

Introducing SPR to Sutton's lab

Sutton quickly turned to surface plasmon resonance technology with the help of a RO1 grant reviewer. "When I was writing an RO1 proposal, a reviewer came back and said it's too bad you don't have SPR to do these experiments," said Sutton. The use of SPR technology was becoming widespread at that time. Once Sutton learned more about the technique, he found it would be a perfect method to study his interactions.

Sutton found that SPR technology measured sensitive kinetics in real time and didn't require tagging or other protein modifications. His lab acquired an early Biacore SPR instrument and, purchased a Reichert 7500DC in 2011. "SPR has proven to be the only technique that's sensitive enough, quantitative enough, and conducive to not labeling proteins," Sutton said. For multiple, relatively weak interactions like those influencing DNA repair enzymes, these qualities have been essential. "SPR in single format gives you information about stoichiometry and kinetics, without modifying biomolecules. It allows detection of small effects on binding constants," he added.

Sutton Determines Reichert Systems are Better for His Lab Than Biacore

In anticipation of using SPR more frequently, Sutton was looking to upgrade his Biacore X with an instrument that was automated, performed better and had significantly lower running costs. Sutton found that the Reichert SR7500DC system fulfilled these key requirements while also meeting his budget. In addition, Sutton felt comfortable with Reichert specifically because of Reichert's century-long legacy in manufacturing robust, high quality instruments and with Reichert's expert SPR team of Ph.D. application scientists. The Reichert SR7500DC system remains an essential instrument in Sutton's lab. Highlighting the advantages of Reichert systems over Biacore's, Sutton notes, "I like the fact that it's an off-the-shelf

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instrument that doesn't require babysitting, like a Biacore. It's less expensive to maintain, and reagents (chips and components) are cheaper. The biggest hurdle for starting SPR was training my people, designing experiments, and using the instrument. Reichert made training incredibly easy and helped in experimental design."

Reichert SPR Helps Sutton Resolve the Mysteries of Complex DNA Repair Kinetics

In his studies of the interactions between proteins and nucleic acids involving DNA repair, Sutton is using the Reichert 7500DC system to examine what are often transient, weak (but vitally important) binding interactions between these molecules.

"It's important to keep in mind that several polymerases have multiple subunits," he said. "When we look at interactions, we pull one piece out of one complex and another piece out of another complex. These interactions are very weak, but very important biologically."

These interactions have also been difficult to look at with other assays. SPR provided quantitative information about stoichiometry, and verified Sutton's hypothesis concerning the effect of DNA mutations.

Further Studies by Sutton Using SPR

In addition to his own lab work, Sutton collaborates with other researchers, including Joseph Loparo at Harvard University, Elba Guerne at McMaster University, and Anthony Burdis at Cleveland State University. Some of the studies Sutton and his colleagues have carried out include:

- Work published in the *Proceedings of the National Academy of Sciences* [1] showed that the DNA polymerase IV molecule in *E. coli* was controlled by another DNA polymerase (DNA Pol III) and that the mutations in structural clefts and rims of DNA Pol IV affected these interactions and the ability of the polymerase complexes to control DNA repair. The study also challenged a prevalent model among geneticists, called the "toolbelt" model that stipulated that two different polymerases simultaneously bound to the same clamp structure on the Pol IV protein. Instead, SPR showed that binding first occurred at just one site, then at different sites at different stages.
- Another paper, in *Nucleic Acids Research* [2], used SPR binding results to show that a single cleft in the "sliding clamp" region of *E. coli*'s DNA polymerase was the point of action for stimulating Polymerase III replications, but, that two cleft structures were needed for releasing the clamp from DNA after replication was finished. The sliding clamp structure tethers replicative DNA polymerases to DNA templates, spurring on the replication (and sometimes, repair) process.

Sutton has additional unpublished SPR data, including studies conducted with the Reichert SPR system which look at these polymerase-polymerase interactions. "The expression level of these polymerases is regulated in response to DNA damage. Most are regulated at the level of transcription as part of an SOS response. If damage cannot be repaired, it is directly replicated over. Sometimes it's replicated accurately, so that repair can use the new strand as a template. Other times, it coordinates the incorrect complementary DNA template, and you have a mixed coding base as a template" Sutton said.

Sutton Concludes that Reichert SPR is the Best Technique for His Research

In summary, Sutton has found that the use of SPR, and Reichert's SR7500DC instrument in particular, has been critical to his research. By using SPR, he can now elucidate the actions of the proteins in question, something he couldn't do with other techniques.

References

[1] "Polymerase exchange on single DNA molecules reveals processivity clamp control of translesion synthesis," by James E. Kath, Slobodan Jergic, Justin M. H. Heltzel, Deena T. Jacob, Nicholas E. Dixon, Mark D. Sutton, Graham C. Walker and Joseph J. Loparo, PNAS, 2014, 111(21), pp. 7647-7652.

[2] "Exchange between Escherichia coli polymerases II and III on a processivity clamp," James E. Kath, Seungwoo Chang, Michelle K. Scotland, Johannes H. Wilbertz, Slobodan Jergic, Nicholas E. Dixon, Mark D. Sutton, and Joseph J. Loparo, Nucleic Acids Res,. 2016, 44(4): pp. 1681–1690.

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