SOUTHWESTERN NEWS

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NIH GRANT ALLOWS UT SOUTHWESTERN RESEARCHERS TO STUDY FAST BIOCHEMICAL REACTIONS

DALLAS — August 12, 1997 — Imagine mixing tiny amounts of proteins and other biological molecules in a thousandth of a second and then studying the reactions that take place in a tenth of a second.

A National Institutes of Health (NIH) grant has enabled UT Southwestern Medical Center at Dallas researchers to purchase equipment that makes possible sensitive measurements of the fastest biological processes.

The NIH awarded a $180,000 Shared Equipment Grant to seven UT Southwestern investigators to obtain state-of-the-art rapid mixing and optical monitoring instruments, available to only a few medical centers in the country. The competitive funding is given to scientists who show they can make full use of a sophisticated and expensive laboratory instrument.

"No one of us could justify spending that kind of money to purchase this equipment for any individual project, but a shared facility of this kind is a wonderful asset to our research," said Dr. Elliott Ross, professor of pharmacology and holder of the Greer Garson and E.E. Fogelson Distinguished Chair in Medical Research.

"These instruments let us look at the rates and progress of fast biochemical reactions. By fast, I mean from less than one millisecond to five seconds. This means that we can study the details of how these reactions happen and the chemical nature of their intermediates. For instance, when a light flashes, how fast does the amplifying biochemistry in your retina record that information? How does it work? With this machine, you can actually determine the rates of those reactions," he said.

The instrument is based on four computer-controlled syringes. The syringes

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independently — and very quickly — mix reagents by injecting them into a series of three tiny mixing chambers, each only 15 microliters in volume (five ten-thousandths of an ounce). After mixing, the instrument can do two types of analyses. In the first, called stopped-flow, the reaction mixture is driven into a small quartz chamber, where several different optical techniques can be used to follow the course of the reaction. In the second, called quench-flow, the reaction is stopped at precise times, and the mixture is collected for chemical analysis.

The versatility of the new instrument is crucial for its shared use. Stopped-flow absorbance measurements — looking at colored reaction intermediates — can be used to follow complex enzyme reactions. Stopped-flow circular dichroism, which monitors molecular shape, can follow the rapid folding of a protein into its active conformation. Stopped-flow fluorescence can measure the binding of proteins to each other, changes in their structures and many other parameters. Quench-flow is used when no optical probe is available.

To obtain such versatility, the UT Southwestern researchers worked with the manufacturer, BioLogic S.A., of Grenoble, France, to combine and adapt the necessary components. Specifications on the final order were six pages long and included several custom-designed items.

One UT Southwestern scientist sharing this machine, Dr. Margaret Phillips, assistant professor of pharmacology, will use it to study reactions catalyzed by several enzymes in trypanosomes, the parasites that cause sleeping sickness and Chagas' disease. Dr. Julian Peterson, professor of biochemistry, will use stop-flow spectroscopy to study the functions of P450s, a large class of enzymes that can degrade drugs, synthesize hormones and inactivate toxins. Dr. Philip Thomas, assistant professor of physiology, and Dr. Elizabeth Goldsmith, associate professor of biochemistry, will use circular dichroism to study how proteins fold and how regulators of enzyme action change the structure of their enzyme targets.

Ross and his group study signaling proteins that convey information from drug and neurotransmitter receptors to intracellular regulatory proteins. They will use quench-flow

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measurements to determine how stimulatory and inhibitory signals combine on the millisecond time scale to organize incoming signaling pathways.

"We are trying to measure these individual processes and how they are regulated," Ross said. "Determining the real turn-on and turn-off rates and what regulates the speed of these reactions has not really been done before.

"Where and when a response turns on and turns off is essential information. For instance, when you read, you are taking in a lot of light, but it's the cut-off between black and white that is important; when you hear a voice, the changes convey the information."

Ross said that stop-flow instruments have existed for 30 years, but previous ones were inefficient. They were too slow — the fastest could not measure reactions faster than about 20 milliseconds — and they required too much reagent, often 1,000 times more than is needed now.

"Five years ago, I wouldn't have dreamed of doing this kind of experiment because the equipment wasn't up to it," he said. "We couldn't possibly make enough enzyme or get enough sensitivity."

Other UT Southwestern researchers sharing in the grant are Dr. Joseph Albanesi, associate professor of pharmacology; Dr. James Stull, chairman of physiology and holder of the Fouad A. Bashour Chair in Physiology; and Dr. Clive Slaughter, associate professor of biochemistry and Howard Hughes Medical Institute investigator.

Ross said the researchers on the grant will have priority use of the equipment, but other NIH-funded UT Southwestern scientists will be able to use the instruments as well.

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