Throwing new light on kidney function

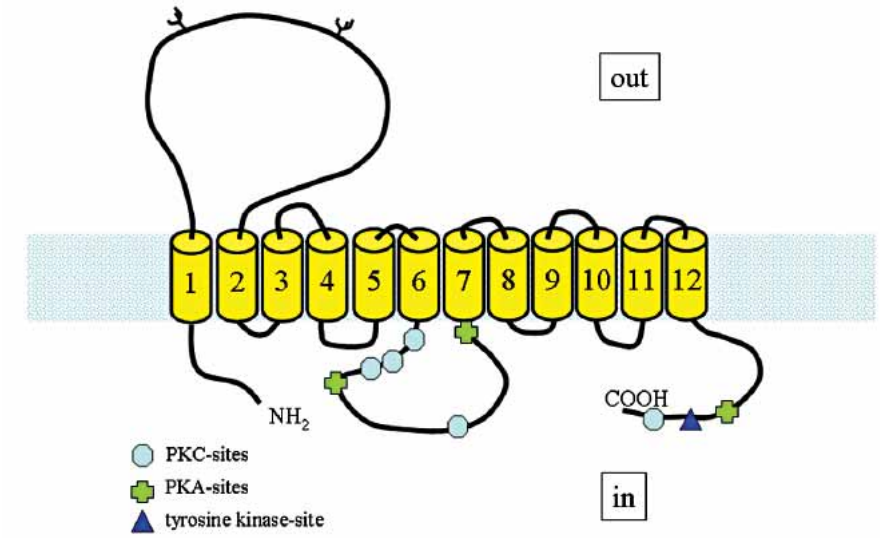
The Experimental Nephrology laboratory at University Hospital Münster is using an Infinite® M200 microplate reader to help investigate kidney function. The group is researching regulation of cell membrane transport, using fluorescence techniques to better understand uptake of pharmaceuticals and organic cations via membrane proteins.

The Experimental Nephrology group at University Hospital Münster

The Experimental Nephrology laboratory, part of the University Hospital Münster, Germany, has been studying function and regulation of the kidneys for over 15 years. The laboratory's work focuses on investigation of membrane transporter proteins, under both normal and pathological conditions, as well as the role of these proteins in causing nephrotoxic drug effects. Current projects within the laboratory include research into ion channels, osmoregulation and organic cation transport, using animal and human tissue models with a variety of cellular and molecular biology, electrophysiology and fluorescence imaging techniques.

The group is particularly interested in the regulation of organic cation transport across the cell membrane, as this process has been implicated in several disease states and is responsible for accumulation of many pharmaceutical compounds within the kidneys. Dr Giuliano Ciarimboli, deputy head of the Experimental Nephrology group, explained. "Some organic cations cannot freely permeate the cell membrane, and are taken up by cells through membrane proteins known as organic cation transporters (OCTs). OCTs are highly expressed in the basolateral membrane of the proximal tubules in the kidneys, and are responsible for the uptake of many endogenous cations, including dopamine, histamine and several neurotransmitters. However, these proteins can also transport many drugs – such as the chemotherapy drug cisplatin – into the kidneys, with nephrotoxic consequences. Our group is studying the function of these proteins, using various techniques to better understand how they are regulated, and investigate potential strategies to prevent the harmful side effects many drugs have on kidney function."

"We have been investigating basolateral membrane transport of organic cations since 1994, using a complex method developed in house. This technique requires an inverted fluorescent microscope to follow the uptake of a marker – ASP^+ (4-(4-dimethylaminostyryl)-N-methylpyridinium) – via a shift in the emission wavelength of the dye. Although this method is excellent for imaging of



Proposed secondary structure of the rat organic cation transporter 1, showing the 12 transmembrane domains and the big intracellular loop containing several potential phosphorylation sites for PKC, PKA and tyrosine kinase.

Ute Neugebauer with the Infinite M200

tissue sections, it is time consuming and unnecessary for study of cell cultures in which cation transport is delocalised, and so we began exploring alternative techniques. Our main goals were to reduce the cost of experiments, as the microscope set-up requires around 500 ml solutions of media and reagents, and increase our throughput capacity for these studies. Performing experiments in a microplate format addresses both these issues, and Ute Neugebauer, a technician in the laboratory, has been instrumental in developing a new method based on a Tecan Infinite M200 microplate reader."

"In this protocol, cells are seeded onto a 96-well microplate and incubated until confluence is achieved. ASP^+ is then added by the Infinite reader's injector module, and uptake is monitored by a shift in fluorescence output from 550 to 590 nm. This allows many experiments to be performed in parallel, with a full set of controls, using a reaction volume of just a few hundred microliters per well. To characterize the performance of this method, we conducted several studies using known substrates of OCTs, such as tetraethyl ammonium and quinine, to competitively inhibit ASP^+ uptake. The Infinite's dual injector option allows ASP^+ to be injected together with another substrate of OCT for rapid determination of IC_{50} values. The microplate technique shows very good correlation with the microscopy-based technique, achieving near identical inhibition curves. The dual injector allows us to very quickly determine if a compound of interest affects ASP^+ transport by OCT proteins. Only compounds showing an interaction are selected for further study, saving both time and money. We have used this strategy to investigate acute regulation of OCTs by specific kinases, injecting known activators or inhibitors of these kinases at variable times before ASP^+ addition."

"Since acquiring our Infinite reader, we have extended its use to several other projects, including measurement of calcium uptake and intracellular pH. We are also using the instrument to study changes in cell volume through osmoregulation, using a method based on the fluorescence of calcein. Tecan has been very supportive of our work here, and we always receive expert assistance when we are developing new techniques."

To find out more on Tecan's Infinite 200 microplate readers, visit **www.tecan.com/infinite200**