Chemical & Physical Sciences
UNIVERSITY OF TORONTO
MISSISSAUGA

# COLLOQUIUM

**TUESDAY, FEBRUARY 19TH, 2013**
**12:00 P.M. (SHARP) – 1:00 P.M.**
**IB270**

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# Rikard Blunck

*Université de Montréal*

## " Probing structure-function relations of potassium channels and pore-forming toxins using fluorescence spectroscopy"

Voltage-gated ion channels are responsible for the generation of action potentials in our central nervous system, and defects in genes encoding for the channels lead to severe familial diseases such as epilepsy, cardiac arrhythmias and congenital deafness. While crystal structures exist for the open state of the channel, for the closed state we still rely on molecular dynamics simulations. Crystal structures also do not provide any structural dynamic information on the kinetics of the proteins' movements. In the presentation different fluorescence spectroscopy techniques to obtain structural information as well as kinetics on ion channels will be introduced. We used *lanthanide-based resonance energy transfer* (LRET) to determine atomic-scale distances for the open and closed state of the channel. Using this data in combination with molecular dynamics simulations, we were able to reconstruct the movement on the internal side of the channel. In *single molecule fluorescence spectroscopy* measurements, we monitored the relative movement of the four subunits in order to analyze their cooperative behaviour. These channels were then reconstituted in planar lipid bilayer in order to correlate single channel current and fluorescence changes. We employed the *single subunit counting* technique to determine the composition of heteromerically expressed ion channels and to follow the oligomerization of pore-forming toxins. In order to optimize accuracy and speed of single subunit counting, we developed an automated analyzes program that extracts the composition of membrane proteins even from noisy total internal reflection data obtained from mammalian cells.