COLLOQUIA IN CELLULAR SIGNALLING

Venue: Medical University Vienna, Center for Physiology and Pharmacology,
Institute of Pharmacology, Waehringerstrasse 13a, 1090 Vienna, "**Leseraum**".
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Wednesday 13.05.2015 11:00 s.t. **Rikard Blunck** (host: W. Sandtner)
Université de Montréal
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"Probing Structure Function Relations of Ion Channels Using Fluroescence Spectroscopy"

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Fluorescence spectroscopy, and in particular voltage-clamp fluorometry, i.e. the simultaneous measurement of structural rearrangements via fluorescence and function via electrophysiology, has proven very powerful to probe the molecular mechanisms underlying ion channel functioning. Using Lanthanide-based resonance energy transfer (LRET), we determined distances in the closed state of Kv channels, that allowed us to suggest a closed state model for voltage-gated potassium (Kv) channels. However, these results are restricted to static structures. We used voltage-clamp fluorometry (VCF) to study the dynamics of conformational changes in Kv channels. While these were previously restricted to sites externally accessible, our introduction of fluorescent unnatural amino acids (fUAAs) to VCF, allows to probe any position in the protein even cytosolic or buried ones. We also used single-molecule voltage-clamp fluorescence imaging to study the oligomerization of single KcsA channels while simultaneously measuring channel activity. Finally, we developed an automated algorithm to analyze single subunit counting data obtained from photobleaching tagged proteins expressed in mammalian cells.