COLLOQUIA IN MEMBRANE TRANSPORT

Venue: Technical University Vienna, Wiedner Hauptstr. 8, 1040 Vienna,

Tower B, yellow part, 2. floor, Lecture Hall 3

(Harald Sitte, Tel.: (01) 4277-64123, harald.sitte@meduniwien.ac.at,
Gerhard Schütz, Tel.: 43 (1) 58801 - 134 80, schuetz@iap.tuwien.ac.at)

Friday 15.06.2012 14:00 s.t. **Max Ulbrich** (host: Gerhard Schütz)

Centre for Biological Signalling Studies
University Freiburg
Habsburgerstr. 49
79104 Freiburg
Germany

"Single molecule imaging of membrane protein interactions and dynamics in living cells"

Max Ulbrich (max.ulbrich@bioss.uni-freiburg.de)

Abstract.
Due to improved equipment and a constantly increasing variety of fluorescent proteins and organic dyes, single molecule techniques developed from a topic for specialists in physics to a tool for many biological applications. One method that our group is helping to establish is the counting of GFP bleaching steps from subunits of oligomeric proteins in order to determine their multimerization state and the rules of their assembly. As an extension of the subunit counting approach, we use two colors to investigate more complex interactions of several different subunit types with each other.
Recently, we analyzed the stoichiometry of synaptic ion channels and receptors because they are immobile in the membrane of Xenopus oocytes and therefore can be imaged easily. However, the subunit counting approach is more difficult for proteins that display high mobility in the cell membrane, because they show stronger fluctuations in intensity and can disappear from the field of view. A particular challenge is the analysis of GPCR interactions because the stoichiometry is not fixed, but there exists a finely tuned equilibrium between monomers and dimers that is postulated to be activation dependent for several clinically highly relevant receptors. Specific and effective labeling with multiple color tags is a prerequisite for the quantitative determination of this interaction, and we show how to tackle the problem of expressing multiple proteins at very low but equal levels in mammalian expression systems.