Tary Ca$^{2+}$ sub-optimal or optimal ligand conditions. Furthermore, in two-channel current ligand conditions is independent of whether the IP3R channel is in single or logical ranges of free Ca$^{2+}$.

The ubiquitous inositol 1,4,5-trisphosphate (IP3) receptor (IP3,R) channel, localized primarily in the ER membrane, releases Ca$^{2+}$ into the cytoplasm upon binding IP3, generating and modulating intracellular Ca$^{2+}$ signals that regulate numerous physiological processes. Together with the number of channels activated and the open probability ($P_o$) of the active channels, the size of the unitary Ca$^{2+}$ current ($i_{Ca}$) passing through an open IP3,R channel determines the amount of Ca$^{2+}$ released from the ER store, and thus the amplitude and the spatial and Ca$^{2+}$ signals generated in response to extracellular stimuli. Despite its significance, $i_{Ca}$ for IP3,R channels in physiological ionic conditions has not been directly measured. Here we report the first measurement of $i_{Ca}$ through an IP3,R channel in its native membrane environment under physiological ionic conditions. Nuclear patch-clamp electrophysiology with rapid perfusion solution exchanges was used to study the conductance properties of recombinant homotetrameric rat type 3 IP3,R channels. Within physiological ranges of free Ca$^{2+}$ concentrations in the ER lumen ([Ca$^{2+}]_{ER}$), free cytoplasmic [Ca$^{2+}]$ ([Ca$^{2+}]_{cyt}$) and symmetric free [Mg$^{2+}$] ([Mg$^{2+}]$), the $i_{Ca}$-[Ca$^{2+}]_{ER}$ relation was linear with no detectable dependence on [Mg$^{2+}$]. $i_{Ca}$ was 0.15 ± 0.01 pA for a filled ER store with 500 μM [Ca$^{2+}]_{ER}$.

In sub-optimal ligand conditions, clustered channels gates identically and independently, but with a lower open probability ($P_o$) than lone channels regardless of cluster size. In contrast, in optimal ligand conditions, $P_o$ for clustered and lone channels were the same, but positive cooperative gating was consistently detected in patch-clamp recordings from clusters. Seeking to clarify these observations, we acquired current records from the same channels expressed in the same cell system with identical protocols and ligand conditions. The records were analyzed with the same algorithm to characterize gating behaviors of lone and clustered channels separately. For comparison, all nuclear patch-clamp current records previously acquired under comparable ligand conditions for recombinant rat type 3 IP3,R channels expressed in Xenopus oocytes, endogenous IP3,R channels in isolated mouse IP3,R channels in insect S9F cells were similarly analyzed. We found that rat type 3 IP3,R channels in DT40-KO cells are clustered without exposure to IP3, like all IP3,R channels investigated before. For all IP3,R channels examined, we detected no significant differences between channel $P_o$ of lone and clustered channels, in sub-optimal or optimal ligand conditions. Furthermore, in two-channel current records, the same pattern of channel gating was detected, in both sub-optimal and optimal ligand conditions, with only a small fraction (< 15%) revealing positive cooperative gating behavior. Thus, single channel behavior in all ligand conditions is independent of whether the IP3,R channel is in single or multi-channel patches.

**Motions of the Cell Surface Molecules**

**1376-Pos Board B286**

**Impact of Anomalous Diffusion on Biochemical Kinetics**

Marcel Hellmann, Matthias Weiss, Dieter W. Heermann.

Biochemical reactions ultimately rely on the (repetitive) encounter of two or more molecules. Diffusive transport of the reactive partners not only determines the first encounter but also the subsequent time window during which the proteins stay near to each other and repeatedly collide. Experimental observations have highlighted that diffusion in crowded media like the cell’s cytoplasm or the plasma membrane is strongly anomalous, i.e. the area that is explored by a particle grows less than linearly in time. We show here that anomalous diffusion can dramatically alter the time course of biochemical reactions. In general, the behavior deviates from the classical Smoluchowski result and shows signatures of anomalous, fractal-like kinetics. While single-step reactions are typically slowed down by anomalous diffusion, multi-step reactions (e.g. phosphorylation cascades) can also be enhanced. Hence, biochemical networks in living cells could have emerged in a way to deal with or even exploit anomalous subdiffusion.

**1377-Pos Board B287**

**A new Paradigm in Single-Particle Tracking in Live Cells: Onset of Ergodicity Breaking**

Diego Krapf, Aubrey V. Weigel, Blair Simon, Michael M. Tamkun.

Experimental single-particle tracking (SPT) is extensively used to study the dynamics of membrane proteins and lipids in living cells. Most studies show that molecular motion in the plasma membrane, as well as in the cytoplasm and the nucleus, undergoes anomalous subdiffusion. SPT data is usually analyzed in terms of the temporal mean square displacement because averaging along individual trajectories is more readily available than ensemble averages. However, in some intriguing physical phenomena ergodicity is broken and thus temporal averages do not converge to the ensemble measurements. Here we measured more than 1,000 Kv2.1 potassium channel trajectories in live human embryonic kidney (HEK) cells, analyzed their ergodic and non-ergodic properties and uncovered the physical mechanism underlying their anomalous diffusion pattern. We have labeled Kv2.1 channels with quantum dots (QDs) in HEK cells, imaged the cell basal membrane with total internal fluorescence microscopy and analyzed the individual channel trajectories. We found that the distributions of the two types of averages are clearly different. The temporal average yielded a much broader MSD than the ensemble-average. Nevertheless, the diffusion pattern of these channels follows anomalous subdiffusion in the lag time, i.e. the MSD is sublinear, both for the temporal and the ensemble averages. Our data reveal that two processes simultaneously coexist and only one of them is ergodic. The ergodicity breaking is found to be maintained by a set of anchoring points associated to the actin cytoskeleton. Control experiments indicate these effects are not induced by the quantum dots. These results are accurately modeled by a continuous time random walk on a fractal structure. When the actin cytoskeleton is disrupted, ergodicity is recovered. These experimental observations have direct biological implications in the dynamics of membrane proteins.

**1378-Pos Board B288**

**Single Molecule Tracking Under an External Force Field Created by Amplifying Hydrodynamic Drag with a Nano-Parachute**

Silvan C. Türkcan, Jean-Marc Allain, Michel R. Popoff, Antigoni Alexandrou.

The membrane architecture plays a crucial role in many cellular processes. In this work we track the motion of two membrane receptors in an induced external force field that distorts the thermal equilibrium and gives access to the receptor interaction with the environment. We introduce a scheme that is easier to use and cheaper than optical tweezers and allows multiplexed measurements. Lanthanide-doped nanoparticles (Y0.6Eu0.4VO4) are coupled to two different bacterial pore-forming toxins. Single-molecule tracking (SMT) of receptor-bound labeled toxins in the membrane of MDCK cells reveals that the receptors undergo confined diffusion in stable domains. We take advantage of the hydrodynamic interaction of labeled receptors with a controlled fluid flow within a microfluidic channel to apply a force on the receptors (Figure 1A). The nanoparticle label acts as a parachute and increases the hydrodynamic interaction with the fluid, so that drag induced by convection becomes important. In B), SMT shows mainly elastic displacements of the receptors over distances up to 10 times the confining domain diameter. Once the flow stops, the receptors return to their initial position indicating attachment to the cytoskeleton.