receptors as probes to determine formation and spatial distribution of individual FG-Nups in the native NPC with a spatiotemporal resolution of 9-10 nm and 400 ms. We found that the NPC complex interacts with the central scaffold of NPC and act as the primary selective gate for large single-independent cargoes. On either side of the central barrier, Nup98 and other FG-Nups could function as the secondary selective barrier for incoming molecules. Finally, the conformation of the central selective barriers can be significantly regulated by a major transport receptor Importin β1, but not by the other transport receptors including Importin β2, Cmn1, NTF2 and CAS. 2678-Pos Board B448 Molecular Settings in the Nuclear Pore Complex of Live Cells Francesco Cardarelli1, Luca Lanzano2, Enrico Gratton2.

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Nuclear pore complexes (NPCs) are the gateways for nucleocytoplasmic exchange. Measurements of molecular transport through NPCs may provide valuable information to unravel the mechanism of communication between the nucleus and the cytoplasm. Unfortunately, because single molecules undergo very rapid transport, it is challenging to follow their motion in live cells. We set out to address the nanomechanical basis of pore function in intact cells by a combination of fluorescence correlation spectroscopy (FCS) and realtime tracking of the center of mass of single NPCs. We find the dynamics of the nucleoporin Nup153 to be regulated at the nanoscale level so as to produce rapid, discrete exchange between two separate positions within the NPC. By means of the pair correlation function (PCF) analysis we are able to identify the two components of Nup153 exchange: a fast collapse into compact molecular conformations (cytoplasm-to-nucleus) and a slowly sliver release into extended conformations (nucleus-to-cytoplasam). We demonstrate that this signature activity is directly linked to the functional import of classical transport receptors and cargos. Thus, we propose that the selective gating through intact NPCs is largely powered by spring-like molecular engines.

2679-Pos Board B449 Physical Modeling of the Conformational Dynamics of the Flexible Unfolded Proteins of the Nuclear Pore Complex Anton Zilman1, Michael Opferman2, Rob Coalson2, David Jasnow2, Paul Welch1.

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Selective transport through the Nuclear Pore Complex (NPC) relies on the interactions of the transport factors with the natively unfolded proteins within the NPC channel (the FG nups). Despite recent advances, it is still not fully known how such transport can be mechanistically understood in terms of conformational dynamics of the FG nups controlled by the transport factors. Creation of artificial selective nano-channels functionalized with the FG nups (that mimic the NPC) or other flexible polymeric molecules extends the question of transport selectivity to a more general context. Because many of the details of the conformational dynamics of the FG nups are not directly accessible experimentally on the relevant time scales, computational modeling is an important tool in addressing these questions. We present results of physical modeling of the effects of the transport factors on the conformational dynamics of FG nup-like flexible molecules in various geometries in order to explain experimental observations in vivo and in vitro. We establish which of the aspects of the conformational dynamics of the FG nups are essential for selective and efficient transport, and which are merely byproducts of the diffusion of the transport factors through a polymer-like medium. Finally, we propose a general coarse-grained description of selectivity in NPC-like channels.

2680-Pos Board B450 FRAP Analysis of Nuclear Export Rates Identifies Intrinsc Features of Nucleocytoplasmic Transport Francesco Cardarelli1, Luca Tosti2, Michela Serresi2, Fabio Beltrami2,3.

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Aiming at a quantitative description of carrier-mediated nuclear export in live cells, we fused a prototypical leucine-rich nuclear export signal (NES) to GFP as a model cargo and expressed the fluorescent chimera into live CHO-K1 cells. The relevant parameters of NES-mediated nucleo-cytoplasmic transport were recovered by FRAP following an established theoretical description of kinetic exchanges between the cytoplasm and the nucleus. By our approach we were able to calculate the affinity of the expressed NES for the export machinery and the maximum rate of nuclear export achievable at saturation of endogenous carriers. Remarkably, the maximum export rate resulted similar to previously-determined maximum import rate; additionally, we demonstrated that export is not affected by the co-expression of saturating levels of a fluorescently-labeled nuclear import signal (NLS). Our results reveal the symmetry and dynamic decoupling between active export and import fluxes, thus highlighting the gating properties of single nuclear pores.

Voltage-gated Na Channels II

2681-Pos Board B451 A Proton Leak Current through the Sodium Channel Linked to Mixed Arrhythmia and Dilated Cardiomyopathy Phenotypes Pascal Gosselin-Badourudine1, Dagmar I. Keller2, Hai Huang3, Aurelien Chatelier4, Mohamed Chabine5.

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Cardiac Na⁺ channels encoded by SCN5A gene are essential for initiating heart beats and maintaining a regular heart rhythm. Mutations in these channels have recently been associated to atrial fibrillation, ventricular arrhythmias, conduction disorders and dilated cardiomyopathy (DCM).

We performed a young male patient with a mixed phenotype composed of documented conduction disorder, atrial flutter, and ventricular tachycardia and DCM. Further family screening revealed DCM in the patient’s mother and sister. Because of the complex clinical phenotypes, we screened SCN5A and identified a novel mutation, R219H, which is located on a highly conserved region on the fourth helix of the voltage sensing domain of Na⁺, 1.5. Three family members with DCM carried the R219H mutation. The wild-type (WT) and mutant Na channels were expressed in a heterologous expression system and intracellular pH (pHi) was measured using a pH-sensitive electrode. The biophysical characterization of this mutant channel revealed an unexpected selective proton leak without any effect on the channel’s biophysical properties. This H⁺ leak through the mutated Na⁺, 1.5 channel was not related to the Na⁺ permeation pathway but occurred through an alternative pore. This pore most probably involves a proton wire on the voltage sensor domain. We suggest that an acidification of cardiac myocytes and/or downstream events may cause the DCM phenotype and other electrical problems in affected family members. The identification of this clinically significant H⁺ leak may lead to the development of more targeted treatment.

2682-Pos Board B452 Mutations in SNTA1 and SCN5A Interact to Increase Late Sodium Current in a Patient with Syncpe Roumu Hu1, Bi-Hua Tan1, Carmen Valdivia2, Kate M. Orlandi3, Amber Peterson2, Yang He1, Daniel J. Park1, Jielin Pu3, Jonathan C. Makielski1.

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Alpha-1-Syntrophin (SNTA1) interacts with the C-terminus of the cardiac Na channel SCN5A and binds to neuronal nitric oxide synthase (nNOS) and the cardiac isoform of the plasma membrane Ca-ATPase (PMCA4b). We have reported that mutations in SNTA1 associated with a long QT syndrome patient and sudden infant death syndrome with increased late INa. Here, we characterize Na currents produced by A261V-SCN5A and R800L-SCN5A found in a patient with syncpe.

Comprehensive open reading frame/splice site mutational analysis of SCN5A and SNTA1 were performed using denaturing high performance liquid chromatography and DNA sequencing. We engineered R800L into the most common splice variant of SCN5A and A261V into SNTA1, transfected them in HEK293 cells along with nNOS and PMCA4b and measured late Na current by voltage clamp. The figure shows late INa as a percentage of peak INa increased by both mutations, the effects were additive, and blocked by the NG-nomonoethyl-l-arginine (L-NMMA), an NOS inhibitor. This is only the second SNTA1 mutation in an adult associated with atrial arrhythmias and increase late INa, and it interacts with an SCN5A mutation in an NOS dependent mechanism.