the extracellular region. Rewiring effect of inter-residue interactions in 493R mutant pocket allosterically propagates across the channel resulting in a more stabilized global conformational ensemble of the channel. These findings predict a novel mechanism of ENaC’s constitutive activity, in which changes in local dynamics can affect the relative population of the channel’s active states and its open probability.

2949-Pos Board B379
Role of Threonine 338 in CFTR Gating
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Introduction: The cystic fibrosis transmembrane conductance regulator (CFTR) is a member of the ATP-binding cassette (ABC) superfamily and is essential for proper fluid and ion transport across cell membranes. The gating of CFTR is allosterically coupled to opening/closing of the channel. Recent cytochrome c scanning of CFTR’s TMDs not only identified pore-lining residues, but also suggested molecular motions of the TMDs involved in opening/closing of the “gate”. Since many of the pore-lining residues exhibit clear state-dependent exposure to the aqueous pore, it is predicted that mutations at these positions might affect gating by altering the free energy level of a particular state. T338 was chosen because this pore-lining residue is mostly concealed from the pore in the closed state. We converted T338 to various amino acids and found that the physical properties of the side-chain at this position indeed affect CFTR gating as well as anion conductance. For hydrophilic residues like threonine itself (0.57 ± 0.06, n = 4), serine (0.45 ± 0.02, n = 12) and asparagine (0.38 ± 0.02, n = 6), the larger the side-chain, the higher the Po. In contrast, for hydrophobic ones such as alanine (0.60 ± 0.02, n = 6), isoleucine (0.19 ± 0.03, n = 6) and valine (0.44 ± 0.05, n = 7), the larger the side-chain, the lower the Po. Single-channel kinetic analysis revealed that mutations mainly affect the open time. To exclude possible effects of the mutation on ATP hydrolysis, we introduced some mutations into the E1371S background, whose ATP hydrolysis is abolished. Interestingly, mutations that shorten the open time under the wild-type background also decrease significantly the Po.

Methods and Results: Mutations introduced into the E1371S background decreased the Po compared to the wild-type background. The decrease in Po was significant when mutations were introduced in hydrophilic residues like threonine (0.45 ± 0.02, n = 12), serine (0.38 ± 0.02, n = 6), asparagine (0.38 ± 0.02, n = 6), and hydrophobic residues like alanine (0.60 ± 0.02, n = 6), isoleucine (0.19 ± 0.03, n = 6) and valine (0.44 ± 0.05, n = 7). The success rate of gigaohm seal formation was 37%. Then we recorded single channel currents with a slope conductance of 285.4 pS, which is parallel to the known planar patch clamp recordings. Using our planar patch clamp system, a gigaohm stretch-activated KCa (SAKCA) channel, which is mechanosensitive, for responses of ion channels. Planar electrodes of 100 μm thickness were fabricated using siliconic microwave. Next, we prepared HEK293 cells transfected with the stretch-activated KCa (SAKCA) channel, which is mechanosensitive, for planar patch clamp recordings. Using our planar patch clamp system, a gigaohm seal was achieved with a maximum seal resistance of 10 GΩ. The success rate of gigahm seal formation was 37%. Then we recorded single channel currents with a slope conductance of 285.4 pS, which is parallel to the known SAKCA current. Using scanning electron microscope, we confirmed elongation of the aperture by 37.7% when 50% stretch was applied to the planar electrode. It is expected that a controllable and sufficient stretch stimulus can be applied to the cellular membrane using our newly developed planar patch clamp system.

2953-Pos Board B383
Single-Channel Analysis of the Molecular Pharmacology of the Long QT Syndrome Variant 3
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The Long QT Syndrome (LQTS) is characterized by a prolongation of the QT interval on an ECG and occurrences of ventricular fibrillation, polymorphic ventricular tachycardia, and sudden cardiac death. In patients with LQTS variant 3 (LQT3), mutations in the cardiac sodium channel alpha subunit, Nav1.5, disrupt channel inactivation by multiple mechanisms and can cause a sustained depolarizing current (I_{sust}) sufficient to prolong the ventricular action potential. LQT3 mutant sodium channels are therefore a reliable experimental model for the study of the function and pharmacology of dysfunctional P1P-RKVxF- binding is a prerequisite for dephosphorylation of pSer68-PLM.