THE S4-S5 LINKER OF KCNQ1 CHANNELS FORMS A STRUCTURAL SCAFFOLD WITH THE S6 SEGMENT CONTROLLING GATE CLOSURE.

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In vivo, KCNQ1 α-subunits associate with the β-subunit KCNE1 to generate the slowly activating cardiac potassium current (I\textsubscript{Ks}). Structurally, they share their topology with other Kv channels and consist out of six transmembrane helices (S1-S6) with the S1-S4 segments forming the voltage sensing domain (VSD). The opening or closure of the intracellular channel gate, which localizes at the bottom of the S6 segment, is directly controlled by the movement of the VSD via an electromechanical coupling. In other Kv channels this electromechanical coupling is realized by an interaction between the S4-S5 linker (S4S5\textsubscript{L}) and the C-terminal end of S6 (S6\textsubscript{T}). Previously we reported that substitutions for L353 in S6\textsubscript{T} resulted in channels that failed to close completely. Closure could be incomplete because L353 itself is the pore occluding residue of the channel gate or because of a distorted electromechanical coupling. To resolve this and to address the role of S4S5\textsubscript{L} in KCNQ1 channel gating, we performed an alanine/tryptophan substitution scan of S4S5\textsubscript{L}. The residues with a “high impact” on channel gating (when mutated) clustered on one side of the S4S5\textsubscript{L} α-helix. Hence, this side of S4S5\textsubscript{L} most likely contributes to the electromechanical coupling and finds its residue counterparts in S6\textsubscript{T}. Accordingly, substitutions for V254 resulted in channels that were partially constitutively open and the ability to close completely was rescued by combination with substitutions for L353 in S6\textsubscript{T}. Double mutant cycle analysis supported this crosstalk indicating that both residues come in close contact and stabilize the channel’s closed state.

INTRODUCTION

KCNQ1 (KvLQT1) α-subunits tetramerize to create a voltage-gated K+ (Kv) channel. Like other Kv channels, each α-subunit contains six membrane spanning segments (S1-S6) with a pore loop between the fifth and sixth segment that forms the selectivity filter. The co-assembly with KCNE1 (minK)-subunits generates the channel complex that underlies the native I\textsubscript{Ks} in the heart (1;2). A fundamental property of all Kv channels is their ability to detect a change in membrane potential (Vm) and to respond to this change by opening or closing their activation gate that seals off the ion permeation pathway in a closed configuration (3;4). The channel activation gate is located in the COOH-terminal end of the S6 segment (S6\textsubscript{T}) whereas the voltage-sensing domain (VSD) is formed by the S1-S4 segments with the charged S4 being the main component (5). Substitution of the S4 charges in KCNQ1 perturbed channel gating (6;7) and gating currents that originate from the redistribution of these S4 charges have recently been recorded for the KCNQ-family of Kv channels (8). These data
indicate that as in *Shaker*-type channels, the S1-S4 segment in KCNQ1 forms the channel VSD that reorients upon a change in membrane potential. This VSD reorientation is then translated into channel gate opening or closure through an electromechanical coupling. This coupling mechanism remains poorly defined but several studies suggest an interaction between the S4-S5 linker (S4S5L) and S6T (9-14). The crystal structure of Kv1.2, a mammalian *Shaker*-type K+ channel, indicated that residue contacts at the coupling interface between S4S5L and S6T are mainly hydrophobic, and it was suggested that they serve to transfer the energy from the VSD movement onto the channel gate (15-17). On the other hand, in slowly activating channels such as hERG and HCN channels mainly electrostatic interactions between the S4S5L and S6T have been identified (9-11).

We previously identified in KCNQ1 several S6T residues that are involved in opening or closure of the channel gate (18). In particular, an alanine or a charged residue substitution for L353 precluded normal channel closure and resulted in a constitutively partial open channel. This impaired channel closure could be explained in two ways: 1) the side chain of residue L353 itself forms the cytoplasmic activation gate that seals off the ion permeation pathway or 2) L353 interacts with residues of S4S5L in the closed channel configuration and mutating L353 results in a loosened electromechanical coupling with a failure to close completely. To investigate the latter possibility further, we identified the residues in the S4S5L of KCNQ1 that participate in the gating machinery. These residues group together on one side of S4S5L according to a homology model based on the Kv1.2 structure. By combining specific S4S5L substitutions with L353 mutants we could restore normal channel closure suggesting that these residue pairs interact or come in close contact in the closed channel configuration and are part of the electromechanical coupling in KCNQ1 channels.

**EXPERIMENTAL PROCEDURES**

*Molecular biology*

hKCNQ1 was expressed using a pIRES2-EGFP expression vector (BD Biosciences, San Jose, CA). Mutations were introduced with a PCR reaction using mutant primers and the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). After PCR based mutagenesis, a Pvu I - EcoR I fragment containing the mutation was cut out of the PCR-amplified vector and ligated in hKCNQ1/pIRES2-EGFP to replace the wild-type (WT) sequence. Double-strand sequencing of the exchanged fragment and the adjacent sequence confirmed the presence of the desired modification and the absence of unwanted mutations. Plasmid DNA for mammalian expression was obtained by amplification in XL2 Bluescript cells (Stratagene), and then isolated from the bacterial cells with the endotoxin-free Maxiprep kit (Sigma, St Louis, MO).

**Electrophysiology**

CHO-K1 cells were cultured in Ham F12 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA). The cells were transfected with 6 µg cDNA of WT or mutant KCNQ1 following the fugene transfection method (Roche Diagnostics, Basel, Switzerland). For electrophysiological experiments untagged KCNQ1 constructs were used. 16 h after transfection the cells were trypsinized and GFP fluorescent cells were used for experiments within 12 h. Current recordings were made with a Multiclamp-700B amplifier (Axon Instruments, Foster City, CA) in the whole cell configuration of the patch clamp technique. Experiments were performed at room temperature (20-23°C); current recordings were low pass-filtered and sampled at 2-10 kHz with a Digidata 1322A data acquisition system (Axon Instruments). Command voltages and data storage were controlled with pClamp8 software (Axon Instruments). Patch pipettes were pulled from 1.2-mm borosilicate glass capillaries (World Precision Instruments, inc., Sarasota, Florida) with a P-2000 puller (Sutter Instruments, Novato, CA) and afterwards heat polished. The cells were perfused continuously with a bath solution containing in mM: 145 NaCl, 4 KCl, 1.8 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose, and adjusted to pH 7.35 with...
NaOH. The pipette solution contained in mM: 110 KCl, 5 K$_2$BAPTA, 5 K$_2$ATP, 1 MgCl$_2$, 10 HEPES and was adjusted to pH 7.2 using KOH. Junction potentials were zeroed with the filled pipette in the bath solution. The remaining liquid junction potential was estimated to be 1.7 mV and was not corrected. The access resistance varied from 3 to 9 MΩ without compensation and was below 3 MΩ after whole cell compensation. Experiments were excluded from analysis if voltage error estimates exceeded 5 mV.

**Data analysis**

The holding potential was set to –80 mV and the interpulse interval to at least 15 s. The voltage protocols were adjusted to determine the biophysical properties of WT and mutant channels adequately. Time constants of activation and deactivation were determined by fitting the current recordings with a single or double exponential function. The voltage-dependence of channel activation was fitted with a Boltzmann equation: $y = 1/(1+\exp(-(E-V_{1/2})/k))$, in which $k$ represents the slope factor, $E$ the applied voltage, and $V_{1/2}$ the voltage at which 50% of the channels are activated and referred to as the midpoint potential. Both $V_{1/2}$ and the slope factor $k$ were used to calculate the Gibbs free energy of activation at 0 mV ($\Delta G_0$): $\Delta G_0 = 0.0002389zFV_{1/2}$, with the factor 0.0002389 to express the values in kcal/mol. $\Delta\Delta G_0$ was calculated as $(\Delta G_0^{\text{mutant}} - \Delta G_0^{\text{WT}})$. Standard errors of $\Delta G_0$ and $\Delta\Delta G_0$ were calculated using linear error propagation (19). Results are expressed as mean ± S.E.M. with n the number of cells analyzed.

**Confocal Imaging**

WT and mutant KCNQ1 constructs were tagged with GFP at their carboxy terminus. CHO-K1 cells were grown on cover slips and transfected with 2 µg of WT KCNQ1-GFP or mutant cDNA. 48 hours after transfection, confocal images were obtained on a Zeiss CLSM 510, equipped with an argon laser (excitation, 488 nm) for the visualization of GFP.

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**RESULTS**

**Alanine/tryptophan scanning mutagenesis of the S4S5$_L$ in KCNQ1**

To determine which residues in S4S5$_L$ are involved in KCNQ1 channel gating, we performed an alanine substitution scan combined with selected tryptophan mutations. The S4S5$_L$ of KCNQ1 is mainly hydrophobic with on both ends an arginine residue (R249, R259) (Table 1). All residues from T247 to Q260 were mutated to an alanine to reduce the volume of the side-chain, except in the case of a native glycine. Small residues like G252, S253, V254 and V255 were also mutated to a tryptophan to increase the volume of the side-chain substantially. Alanine and tryptophan perturbation mutagenesis has been used previously to explore secondary structure and protein-facing residues of both water-exposed and membrane-embedded domains in several voltage-gated K+ channels (20-22). WT KCNQ1 channels displayed currents that were characterized by a single exponential time course of activation with an apparent threshold of approximately -40 mV. Upon membrane repolarization, a slow deactivation process was preceded by a transient increase in current, a “hooked tail”, resulting from a rapid recovery of channel inactivation that occurred during the preceding depolarization (Figure 1) (1;2;23).

Based on the impact of the substitution on the channel gating properties compared to WT, the residues were categorized in two groups: (1) “high impact” residue positions where a mutation had a marked effect on channel gating and (2) “low impact” positions where a substitution was well tolerated and resulted in channels with biophysical properties fairly similar to WT KCNQ1. First the effect of the mutation on the voltage-dependence of activation was determined by calculating $\Delta G_0$ and subsequently $\Delta\Delta G_0$ (Table 1, for details on the calculations see Experimental Procedures). To quantify the relevance of the perturbation we used a cutoff value of 1 kcal/mol. Using this cutoff value four mutants (L251A, V255W, H258A and R259A) scored as “high impact” (Figure 1, Table 1) and they all displayed a strong positive shift in the voltage-dependence of activation. Taking these shifts into account, the time constants of channel
opening and closure for R259A were similar to WT, suggesting that this substitution only changed the energy level between the open and closed state ($\Delta G_0$). The other substitutions (L251A, V255W and H258A) altered, besides $\Delta G_0$, also the channel gating kinetics and displayed a more than 3-fold slowing of the activation or both activation and deactivation kinetics. In addition, T247A also resulted in a 3-fold change in activation kinetics. Although T247A had a lower impact on $\Delta G_0$ than the other substitutions it was included in the group of “high impact” residues because of the marked change in activation kinetics.

Although the effect of the W248A and V254A substitutions was not reflected in an altered $\Delta G_0$, they were categorized as “high impact” positions because they resulted in channels with an otherwise abnormal phenotype. The W248A mutant had a voltage-dependence of channel activation with activation and deactivation time constants similar to WT. However, the voltage-dependency of channel inactivation was markedly shifted towards more positive potentials compared to WT (Figure 2). Since channels started to inactivate at potentials where steady-state activation was complete, a crossover of the current traces was observed at more depolarized potentials (from +30 mV to +70 mV). Consequently, the “hooked” tail currents appeared at these potentials only (Figure 2). To explore whether channels might inactivate faster than they opened in the voltage range of -40 to +20 mV, triple pulse protocols were applied. The results showed that this was not the case, suggesting that no inactivation occurred at voltages below +20 mV (supplemental figure 1).

The V254A mutation slowed down channel closure by a 2-fold but did not affect the activation kinetics or the voltage-dependence of channel activation (table 1). Similar to W248A the V254A mutant also displayed pronounced channel inactivation and the steady-state current level at the end of the pulse was comparable to the instantaneous one (Figure 3A). Furthermore, analysis of the instantaneous current amplitude as a function of applied voltage showed constitutive conduction at hyperpolarized potentials (membrane potentials where the channels should be closed); this component (without leak correction) crossed the zero current level at -64 mV which is close to the reversal potential for K$^+$ with the solutions used (Figure 3D). The presence of this constitutive current component was not observed for WT KCNQ1 channels or the other channel mutants. To further investigate the contribution of residue V254 in the channel gating mechanism, we mutated this residue to a negatively charged aspartate (V254E) and a positively charged lysine (V254K). Similar to V254A, the V254E mutation displayed a clear constitutive current component at hyperpolarized potentials (Figure 3). In contrast to the alanine substitution, the voltage-dependent current component of V254E was drastically shifted to more positive potentials compared to WT KCNQ1 (Figure 3, Table 2). A lysine substitution for V254 (V254K) was not expressed at the plasma membrane (not-expressed, NE).

The residues R249, G252, S253, F256, I257 and Q260 belonged to the “low impact” group of substitutions that resulted in mutant channels with gating kinetics similar to WT (Table 1). On the other hand, some mutants like L250A and V254W did not generate any current in the voltage range from -130 mV to +100 mV. To determine what caused the lack of current, these subunits were tagged with GFP and their subcellular localization was determined with confocal microscopy. Figure 4 shows that mutant V254W was retained in the endoplasmic reticulum (ER), indicating that this mutant did not pass the quality control of the ER and did not translocate to the plasma membrane (NE). In contrast, mutant L250A was located at the plasma membrane indicating that the surface expression of this mutant channel was not significantly affected. Rather, the mutation caused a severe disturbance of the gating process rendering the mutant channels non-functional (NF) or stabilized the closed channel configuration such that depolarizations up to +110 mV were insufficient to open the mutant channels. Therefore, L250A was also categorized as “high impact”.

**Restoring channel closure of S4S5$_L$ and S6$_T$ mutants by combination.**

Mutating residue V254 in S4S5$_L$ to an alanine resulted in a channel with a deactivation
failure leading to a constitutive current component (Figure 3). This phenotype was reminiscent of the effect of a similar alanine substitution for residue L353 in S61 (18). If a disrupted coupling is the cause for the partial open phenotype of the L353 mutants, then V254 would be a good candidate to be the interacting partner of L353 in the closed configuration. To explore this possibility we combined the L353A mutation with a leucine substitution at position V254 (V254L), testing whether the increase in side-chain volume at position V254 might compensate for the loss at L353. Indeed, the V254L+L353A double mutant resulted in functional channels that did not display a constitutively current component at hyperpolarized potentials indicating that the channels could close completely (Figure 5). However, the voltage-dependence of activation that was markedly shifted towards positive potentials compared to WT KCNQ1 and both individual mutations V254L and L353A (Table 2). In addition, the activation kinetics were 10-fold slower compared to WT even after correcting for the shifted voltage-dependency, while the deactivation time constants were similar to WT. Thus, the effect of the L353A mutation on pore closure could be rescued by increasing the side-chain volume at residue position V254 in S4S5L. Furthermore, a double mutant cycle analysis for V254L+L353A gave a ∆ΔG0 value of 0.98 kcal/mol. This value is close to the cutoff value of 1 kcal/mol indicating that, besides the rescue of the partial open phenotype, both residues are also coupled in energetic terms.

To investigate further the possible coupling between V254 and residue L353 of S61 in the closed state, we attempted to crosslink both residues using cysteine substitutions. While the L353C mutation was functional, the double mutant V254C-L353C construct displayed impaired trafficking (Figure 4). Since this precluded the crosslinking approach, we combined the different charged aspartate and lysine substitutions at positions V254 and L353 in the hypothesis that a complementary charge at position 254 might rescue the charge mutation at 353. Unfortunately, both double combinations V254E+L353K and V254K+L353E were not tolerated and subcellular localization indicated that the channels did not reach the plasma membrane. Since combining the charge substitutions of V254 and L353 was inconclusive, we investigated whether other positions might rescue the partial open phenotype of the L353E mutant. Therefore we introduced in S4S5L a lysine at the position one helical turn up- or downward with respect to V254. The double mutants L250K+L353E and H258K+L353E resulted in functional channels that displayed robust current activation which was not the case for the individual L250K mutant that was non-functional (NF). Furthermore, introduction of positive charges in S4S5L rescued L353E’s failure to close completely and both double mutants L250K+L353E and H258K+L353E did not display a constitutive current component at hyperpolarized potentials (supplemental figure 2). Thus, in case of the double mutant L250K+L353E, both non-functionality (L250K) and the failure to close completely (L353E) were rescued by the combination. Assuming that L250K alone stabilizes the closed state such that the threshold for channel opening is above +110 mV, double mutant cycle analysis then results in a ∆ΔG0 value of at least -3.0 kcal/mol indicative for both residues being coupled. However, while the H258K mutation rescued the constitutive partial open phenotype of L353E, the double mutant cycle analysis yielded a ∆ΔG0 value of only 0.07 kcal/mol for the H258K+L353E combination. This indicates that both residues are not energetically coupled and affect the same rate-limiting or consecutive transition step(s) independently (24).

**DISCUSSION**

*The S4S5L of KCNQ1 adopts an α-helix and constitutes part of the electromechanical coupling*  

In this study, we performed an alanine/tryptophan perturbation scan of the S4S5L in KCNQ1 to determine the relevant residues in channel gating. The S4S5L is in KCNQ1 largely hydrophobic with both ends marked by a positively charged arginine residue (R249 and R259). Alanine substitutions for these two flanking residues have been described previously (6) and our observations are in agreement with this study. The R249A mutant appeared to have no effect on channel gating whereas the R259A
mutation shifted the voltage-dependence of activation towards positive potentials by about +30 mV (Table 1). Besides this R259A mutation, we identified several other residues that altered channel gating upon mutation and categorized them in 2 groups; (1) residue positions that were defined as having a “high impact” on channel gating when mutated (T247, W248, L250, L251, V254, V255, H258 and R259) and (2) residues that tolerated substitutions well and were therefore marked as having a “low impact” (R249, G252, S253, F256, I257 and Q260).

On an α-helical wheel representation, the “high” and “low” impact residues each cluster at opposite sides of the helix (Figure 6). Such a separation pattern is a strong indication for an α-helical structure of the S4S5 L in KCNQ1, similar to the secondary structure proposed for the related Shaker-type Kv channels (15;25). Interestingly, known LQT1 disease mutations in this segment appear to target the “high impact” residues (W248, L250, L251, V254, H258, and R259) (26-32), further strengthening the idea that this side of S4S5L constitutes an important part of the gating machinery.

Contacts between the S4S5L and S6T stabilize the closed channel conformation

The crystal structure of rKv1.2 showed that the S4S5L of Shaker-type K+ channels adopts an amphipathic α-helix that runs parallel to the lipid/cytoplasmic interface and is positioned over S6T of the same α-subunit (15;16). In Shaker-type Kv channels the direct contacts between S4S5L and S6T are mainly of a hydrophobic nature and flanking non-interacting residues serve a stabilizing role by orienting S4S5L and S6T correctly (33). On the other hand, in the slowly gating hERG and HCN channels, electrostatic interactions between S4S5L residues and S6T have been identified (9-11). In KCNQ1, direct electrostatic interactions seem unlikely as the “high impact” side on the S4S5L is mainly hydrophobic. Furthermore, we showed previously that the residues in the S6T that altered channel gating upon mutation are all uncharged residues (18). Therefore, the contacts between S4S5L and S6T are most likely of a hydrophobic nature in KCNQ1, similar to the other fast activating Shaker-type Kv channels.

A previous substitution scan of S6T in KCNQ1 showed that mutating L353 to an alanine or a charged residue resulted in a channel that failed to close completely. We observed a similar phenotype for the V254A mutation in this study. Two different mechanisms could explain the constitutive partially open phenotype of the L353 mutations: 1) residue L353 forms the cytoplasmic activation gate that seals off the ion permeation pathway or 2) L353 comes in close proximity to residues of the S4S5L in the closed channel configuration and mutation of L353 results in a loosened electromechanical coupling by disrupting subdomain contacts (18). Here we show that mutations at position V254 resulted in a similar phenotype and, more importantly, that the failure to close completely was rescued by combination (double mutant V254L+L353A). Furthermore, double mutant cycle analysis was also suggestive for both residues being coupled. These results are in support of the second hypothesis, suggesting that V254 is the interacting partner of L353 and that their contact (which would be of a hydrophobic nature) stabilizes the closed state in the WT channel.

The electromechanical coupling consists out of multiple S4S5L–S6T interactions.

To interpret the results on a molecular level, we used the recently published KCNQ1 3D homology models for both open and closed channel configurations (34). In the closed state model, the “high impact” positions L250, V254 and H258 are indeed located at the contact interface with S6T (Figure 7). Furthermore, residue V254 comes close enough to residue L353 to allow for VDW interactions (34). This proximity between V254 and L353 in a closed channel conformation fits with the hypothesis that the interaction of both residues stabilizes the closed state. On the contrary, in the open state model, the distance between V254 and L353 increases and becomes too large for both residues to maintain a physical contact. The orientation of both residues in this model is similar to our previously described homology model (18) and L353 points downward to the intracellular mouth of the permeation pore.
Apparently, the residues of S4S5_L that contact S6_T in the open conformation appear to be the same as those in the closed conformation, namely L250, V254 and H258. Although caution is needed with interpretations of homology models, this suggests that the side on S4S5_L that makes contact with S6_T is largely the same in both the open and closed states whereas this does not hold for S6_T that displays a different orientation. Consequently, the electromechanical coupling between S4S5_L and S6_T relies on different residue contacts for the closed and open channel conformations.

The observation that H258K and L353E are not energetically coupled indicates that they do not interact directly but that they affect the rate-limiting transition or consecutive gating steps independently (24). This would mean that both residues have different residue counterparts during the transition from the closed to the open state. For L353 the most likely interaction partners are L250 and V254. However, the fact that H258K could rescue L353E’s failure to close completely suggests that there is a certain degree of flexibility in the coupling. To explain these results mechanistically, we propose that introduction of a negative charged residue at position 353 (L353E) is not favored by its normal hydrophobic interacting partners (L250 and V254). Thus, the 353E side-chain adopts most likely a different orientation, thereby slightly changing the S6_T position preventing full channel closure. Introducing a positive charge (H258K) in S4S5_L would by itself affect the position of S4S5_L and as H258K comes in the vicinity of L353E (Figure 7) the imposed structural rearrangement is apparently such that it compensates for the disruption of L353E. The idea that the S4S5_L-S6_T coupling consists of multiple contacts that have a certain degree of flexibility is quite conceivable for moving parts of the channel protein. Indeed a rather loose S4S5_L-S6_T coupling has been suggested by Choveau et al. (companion manuscript): they showed that peptides containing the S4S5_L or S6_T sequence fragments compete for the channel’s own S4S5_L or S6_T site. This suggests that the S4S5_L can be compared to a ligand that binds to S6_T, and locks the channel gate in the closed conformation. Consistent with our results, S4S5_L peptide fragments were effective when they contained all the “high impact” residues L250, V254 and H258, shown here to contact the S6_T in the closed channel conformation. Likewise, only the S6_T fragments containing residue L353 were effective. The observation that disturbing this interaction results in a constitutive partial open phenotype fits the coupling mechanism in which the binding of S4S5_L to S6_T stabilizes the closed state (companion manuscript). A dynamic coupling between the S4S5_L and S6_T has also been recently suggested for the closed state inactivation mechanism of Kv4.2 channels (35). In this case it was proposed that reaching closed state inactivation was due to an uncoupling of the S4S5_L from S6_T.

**KCNQ1 mutations have the tendency to impair channel closure and, at the same time, to enhance the inactivation process.**

The behavior of the V254A mutation is similar to what has been reported for substitutions at position V310 that result in a reduction of side chain volume. V310 is located at the base of the selectivity filter and mutating it to a glycine or an alanine resulted in markedly enhanced inactivation and in the inability to close completely, similar to the phenotype of V254A (36). In case of V310, the impaired channel closure probably involves contacts with other S5 and S6 residues. The observation that the S4S5_L mutation V254A resulted in a similar phenotype and destabilized the electromechanical coupling in the closed state, shows that several KCNQ1 mutations that affect the overall gating process have a tendency to result in an open channel conformation. The study by Choveau et al. (companion manuscript) shows that the S4S5_L-S6_T interaction indeed stabilizes the closed state. This is comparable to the observations in hERG channels that suggest that the interaction between the S4S5_L and S6_T stabilizes the channels closed state (10;11).

Many Kv channels display C-type inactivation and it has been shown to be a process that involves conformational changes at the level of the selectivity filter (37-41). At least two mechanisms have been proposed for constriction of the selectivity filter: (1) a reorientation of the VSD (42;43) or (2) a rearrangement in S6 after channel opening that is transmitted to the
selectivity filter destabilizing its conducting conformation (41). Possibly, both pathways exist and depending on the type of Kv channel one predominates. In case of the V310 mutation that locates at the base of the selectivity filter, the enhanced inactivation can be caused by directly destabilizing the conducting state of the selectivity filter. In case of the S4S5L mutations at position W248 and V254, the origin of the pronounced inactivation process is less clear. In both homology models for the open and closed channel conformation, residues L250, V254 and H258 are facing the bottom part of the S6 segment whereas other “high impact” residues W248, L251 and V255 are oriented towards the lipid-membrane interface. If this orientation is correct, this suggests that substitutions at the latter positions result in altered gating kinetics by disturbing the anchoring of the S4S5L to the plasma membrane. However, caution is needed with this interpretation since S4S5L might be oriented differently with respect to S6TT in the open and closed conformations, making the contact interface with the S6TT quite broad (on the α-helical wheel representation in Figure 6 stretching from L250 to R259). In case of the W248A mutation, the impact was mainly on the voltage-dependency of inactivation. If the inactivation process in KCNQ1 involves a S4 movement, this would mean that the S4S5L reorientation restricts the movement of the voltage sensing domain. On the other hand, the V254A mutation that, like W248A, induces an enhanced inactivation rate but disturbs the coupling with S6TT supports the idea that inactivation is linked to reorientations within the S6 segment itself.

In conclusion, by performing a residue substitution scan of the S4S5L region in KCNQ1, we established that residue V254 of the S4S5L comes in close proximity to L353 in S6TT upon channel deactivation, stabilizing the closed conformation. In addition to this specific residue pair, the electromechanical coupling is most likely formed by multiple additional contacts which in concert directly influence gate opening and closure, and indirectly also influence the inactivation process of KCNQ1 channels.
REFERENCES

1. Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., and Romey, G. (1996) Nature 384, 78-80

2. Sanguinetti, M. C., Curran, M. E., Zou, A., Shen, J., Spector, P. S., Atkinson, D. L., and Keating, M. T. (1996) Nature 384, 80-83

3. del Camino, D. and Yellen, G. (2001) Neuron 32, 649-656

4. Liu, Y., Holmgren, M., Jurman, M. E., and Yellen, G. (1997) Neuron 19, 175-184

5. Bezanilla, F. (2008) Nat. Rev. Mol. Cell Biol. 9, 323-332

6. Panaghie, G. and Abbott, G. W. (2007) J. Gen. Physiol 129, 121-133

7. Shamgar, L., Haitin, Y., Yisharel, I., Malka, E., Schottelndreier, H., Peretz, A., Paas, Y., and Attali, B. (2008) PLoS. ONE. 3, e1943

8. Miceli, F., Cilio, M. R., Taglialetela, M., and Bezanilla, F. (2009) Channels (Austin.) 3, 274-283

9. Decher, N., Chen, J., and Sanguinetti, M. C. (2004) J. Biol. Chem. 279, 13859-13865

10. Ferrer, T., Rupp, J., Piper, D. R., and Tristani-Firouzi, M. (2006) J. Biol. Chem. 281, 12858-12864

11. Tristani-Firouzi, M., Chen, J., and Sanguinetti, M. C. (2002) J. Biol. Chem. 277, 18994-19000

12. Lu, Z., Klem, A. M., and Ramu, Y. (2002) J. Gen. Physiol 120, 663-676

13. Prole, D. L. and Yellen, G. (2006) J. Gen. Physiol 128, 273-282

14. Batulan, Z., Haddad, G. A., and Blunck, R. (2010) J. Biol. Chem. 285, 14005-14019

15. Long, S. B., Campbell, E. B., and MacKinnon, R. (2005) Science 309, 897-903

16. Long, S. B., Campbell, E. B., and MacKinnon, R. (2005) Science 309, 903-908

17. Pathak, M. M., Yarov-Yarovoy, V., Agarwal, G., Roux, B., Barth, P., Kohout, S., Tombola, F., and Isacoff, E. Y. (2007) Neuron 56, 124-140

18. Boulet, I. R., Labro, A. J., Raes, A. L., and Snyders, D. J. (2007) J. Physiol 585, 325-337

19. Yifrach, O. and MacKinnon, R. (2002) Cell 111, 231-239

20. Hong, K. H. and Miller, C. (2000) J. Gen. Physiol 115, 51-58

21. Li-Smerin, Y., Hackos, D. H., and Swartz, K. J. (2000) Neuron 25, 411-423
22. Monks, S. A., Needleman, D. J., and Miller, C. (1999) *J. Gen. Physiol* **113**, 415-423

23. Pusch, M., Magrassi, R., Wollnik, B., and Conti, F. (1998) *Biophys. J.* **75**, 785-792

24. Mildvan, A. S., Weber, D. J., and Kuliopulos, A. (1992) *Arch. Biochem. Biophys.* **294**, 327-340

25. Ohlenschlager, O., Hojo, H., Ramachandran, R., Gorlach, M., and Haris, P. I. (2002) *Biophys. J.* **82**, 2995-3002

26. Deschenes, D., Acharfi, S., Pouliot, V., Hegele, R., Krahn, A., Daleau, P., and Chahine, M. (2003) *Can. J. Physiol Pharmacol.* **81**, 129-134

27. Franqueza, L., Lin, M., Shen, J., Splawski, I., Keating, M. T., and Sanguinetti, M. C. (1999) *J. Biol. Chem.* **274**, 21063-21070

28. Wang, Z., Tristani-Firouzi, M., Xu, Q., Lin, M., Keating, M. T., and Sanguinetti, M. C. (1999) *J. Cardiovasc. Electrophysiol.* **10**, 817-826

29. Kubota, T., Shimizu, W., Kamakura, S., and Horie, M. (2000) *J. Cardiovasc Electrophysiol* **11**, 1048-1054

30. Napolitano, C., Priori, S. G., Schwartz, P. J., Bloise, R., Ronchetti, E., Nastoli, J., Bottelli, G., Cerrone, M., and Leonardi, S. (2005) *JAMA* **294**, 2975-2980

31. Itoh, T., Tanaka, T., Nagai, R., Kikuchi, K., Ogawa, S., Okada, S., Yamagata, S., Yano, K., Yazaki, Y., and Nakamura, Y. (1998) *Hum. Genet.* **103**, 290-294

32. Labro, A. J., Boulet, I. R., Timmermans, J. P., Ottschytsch, N., and Snyders, D. J. (2010) *J. Mol. Cell Cardiol.* **48**, 1096-1104

33. Labro, A. J., Raes, A. L., Grottesi, A., Van, H. D., Sansom, M. S., and Snyders, D. J. (2008) *J. Gen. Physiol* **132**, 667-680

34. Smith, J. A., Vanoye, C. G., George, A. L., Jr., Meiler, J., and Sanders, C. R. (2007) *Biochem.* **46**, 14141-14152

35. Barghaan, J. and Bahring, R. (2009) *J. Gen. Physiol* **133**, 205-224

36. Seebohm, G., Westenskow, P., Lang, F., and Sanguinetti, M. C. (2005) *J. Physiol* **563**, 359-368

37. Kiss, L., LoTurco, J., and Korn, S. J. (1999) *Biophys. J.* **76**, 253-263

38. Kurata, H. T. and Fedida, D. (2006) *Prog. Biophys. Mol. Biol.* **92**, 185-208

39. Gibor, G., Yakubovich, D., Rosenhouse-Dantsker, A., Peretz, A., Schottelndreier, H., Seebohm, G., Dascal, N., Logothetis, D. E., Paas, Y., and Attali, B. (2007) *Biophys. J.* **93**, 4159-4172

40. Cordero-Morales, J. F., Cuello, L. G., Zhao, Y., Jogini, V., Cortes, D. M., Roux, B., and Perozo, E. (2006) *Nat. Struct. Mol. Biol.* **13**, 311-318
41. Cuello, L. G., Jogini, V., Cortes, D. M., and Perozo, E. (2010) *Nature* **466**, 203-208
42. Panaghie, G., Purtell, K., Tai, K. K., and Abbott, G. W. (2008) *Biophys. J.* **95**, 2759-2778
43. Olcese, R., Latorre, R., Toro, L., Bezanilla, F., and Stefani, E. (1997) *J. Gen. Physiol.* **110**, 579-589
44. Humphrey, W., Dalke, A., and Schulten, K. (1996) *J. Mol. Graph.* **14**, 33-38

**FOOTNOTES**

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TABLE LEGENDS

**Table 1:** Biophysical characteristics of WT KCNQ1 and S4S5L residue mutants. Values are means ± S.E.M. V_{1/2} is the midpoint of activation and k is the slope of the activation curve fitted with the Boltzmann equation. Time constants were derived from mono-exponential fits to activating or deactivating currents. \( \tau_{+60\, mV} \) is the time constant for activation at the potential V_{1/2} + 60 mV. \( \tau_{-50\, mV} \) and \( \tau_{-100\, mV} \) are the time constants for deactivation at the potentials V_{1/2} - 50 mV and V_{1/2} - 100 mV. *: for the activation of the V255W mutant a double exponential fit was used and the time constant of the fast component is shown (for the slow one see figure 1C). \( \Delta G_0 \) represents the Gibbs free energy of activation at 0 mV. \( \Delta \Delta G_0 \) was calculated as (\( \Delta G_0 \)mutant - \( \Delta G_0 \)WT). \( \Delta G_0 \) and \( \Delta \Delta G_0 \) are expressed in kcal/mol. Standard errors of \( \Delta G_0 \) and \( \Delta \Delta G_0 \) were calculated using linear error propagation in accordance to Yifrach et al (19). The number of cells analyzed is represented by n. NE, not expressed in the plasma membrane. NF, non-functional (but in the plasma membrane).

**Table 2:** Biophysical properties of S4S5L mutants alone and in combination with S6T substitutions. For abbreviations see Table 1. NE, not expressed in the membrane. NF, non-functional. ND, non-determined. $: displayed a partial open phenotype. *: determined at +80 mV. **: determined at -120 mV. ***: determined at -70 mV.

FIGURE LEGENDS

**Figure 1:** Biophysical properties of WT KCNQ1 and mutant channels in the S4-S5 linker region. (A) Representative current traces for the WT KCNQ1 channel and several mutants expressed in CHO-K1 cells. Cells were clamped at a holding potential of –80 mV, and 800 - 2500 ms pulses to voltages between –60 to +80 mV were imposed in steps of +10 mV. Tail currents were recorded by stepping to –40 mV (Note the differences in scale bars). (B) Voltage-dependence of activation. Activation curves were obtained by plotting the normalized tail currents as a function of the pre-pulse potential. The solid lines represent the average Boltzmann fits and were compared to WT (dotted line). Parameters are shown in Table 1. Besides T247A, the other mutations resulted in channels that displayed a voltage-dependency that was shifted to more positive potentials compared to WT. (C) Activation and deactivation time constants derived from mono-exponential fits (bi-exponential in case of V255W) to the raw current traces were plotted as a function of applied voltage. All mutants resulted in markedly slowed activation kinetics.

**Figure 2:** The W248A mutant displays enhanced channel inactivation. (A) Representative current traces for the mutant W248A. The panel on the left shows the current activations obtained with 800 ms depolarization steps and the panel on the right these with 5 s pulses. In each case the voltage protocol used is represented on top. Note the “crossing” of the current recordings at +60 mV (red trace) and +30 mV (blue trace) respectively. Also the “hooked” tail currents, typical for recovery from the inactivated state, only appeared with more depolarized potentials (see black boxed inset). For comparison also a blow-up of the tail currents of WT KCNQ1 are provided in the grey box. (B) Voltage dependence of activation determined by plotting the normalized tail current amplitudes, that were obtained after 800 ms depolarizations (left panel A), as a function of prepulse potential. The solid line represents the average Boltzmann fit for W248A (circles) and the dotted line this of WT (open circles). (C) Normalized current voltage curve obtained by normalizing the steady-state current amplitude after 5s prepulses (right panel A, indicated with arrow). Note that there is no current inactivation in the voltage-range of -40 to +20 mV. However, above +20 mV the channels displayed a pronounced inactivation...
behaviour that resulted in a steady-state current decrease. The apparent inactivation curve had a midpoint of $64.9 \pm 3.2$ mV with a slope of $14.7 \pm 0.8$ mV ($n = 5$).

**Figure 3:** V254 mutants display a constitutive conducting phenotype. (A) Representative current trace for the mutant V254A and V254E with the voltage protocol shown on top. Note the pronounced channel inactivation of the V254A mutant and the instantaneous current component of both mutants. (B) The voltage-dependent component of the V254A mutant displayed a similar voltage-dependency as WT KCNQ1. For V254E the activation curve was at least +70 mV shifted towards positive potentials compared to WT (parameters are shown in table 1 and 2). (C) The activation and deactivation time constants of V254A were slightly slowed compared to WT KCNQ1. Taken into account the positively shifted voltage-dependence of channel activation, V254E displayed markedly faster activation time constants than WT whereas channel closure was slowed. (D) Instantaneous current amplitudes (determined after the capacitive transient) as a function of pulse potential for KCNQ1 WT, V254A and V254E.

**Figure 4:** Subcellular localization of KCNQ1 WT and mutants determined by confocal microscopy. The α-subunits were tagged with GFP at their C-terminal end and typical pictures of CHO-K1 cells expressing the channel complexes are represented on top. To determine the distribution of the GFP fluorescence over the cell, the GFP emission profile at a cross-section of the cell was determined by plotting the light intensity as a function of the cross-section distance. These profiles are shown below with the cross-section itself indicated in the picture above by the red line. Note the increased GFP emission intensity at the cell boundaries in the profile of the KCNQ1 WT channel and the mutants L250A and L250K, highlighting the robust plasma membrane expression of these channels. In contrast, the V254W, V254K and V254C-L353C mutants lack displayed no marked membrane associated fluorescence, and the profile showed that more GFP emission was observed intracellularly. On top of each panel is indicated the total number of cells examined (n) with different independent transfections (#) all confirming the representative GFP-channel pattern.

**Figure 5:** combinations of S4S5L and S6T substitutions
(A) Representative currents for the mutants V254L, L353A and the double mutant V254L+L353A. In case of V254L the instantaneous current level at +70 mV depolarization (gray current trace) is indicated with dashed line. (B) Instantaneous current amplitudes for WT, V254L, L353A and the double mutant V254L+L353A. Note that the double mutant V254L+L353A did not display an instantaneous current component as was the case for the single V254L and L353A mutation.

**Figure 6:** α-helical projection of the S4S5L region in KCNQ1.
A α-helical wheel representation of the S4S5L (top) and the S6T (bottom) sequence. The “high impact” positions in S4S5L are indicated with filled grey circles and include T247, W248, L250, L251, V254, V255, H258 and R259 respectively. The registered LQT1 mutations in S4S5L are represented in bold and apparently involve substitutions of the “high impact” residues. Note that the pattern of these residues concurs with an α-helical structure and cluster on one side of the helix. Since these “high impact” positions alter channel gating upon mutation, this side of the helix presumably interacts with other segments of the KCNQ1 protein presumably the S6T region for which the sequence is shown below. In case of S6T, residue L353 is marked with a grey circle. Note that both helices run anti-parallel with respect to each other.

**Figure 7:** KCNQ1 3D model showing the S4S5L/S6T interactions in both the open and closed state.
Illustrations were produced using the recently published KCNQ1 homology models for both the closed and open state (34), and the program Visual Molecular Dynamics (44). (A) Side view on the pore domain with the closed state on the left and the open on the right. For clarity the front and back subunit were omitted. In cyan the sequence of one subunit displayed in ribbon representation, with all segments besides S6 shown in transparency. The important residues are shown with their solvent accessible surface. The “high impact” residues in S4S5L that according to the model are in close contact to residues in S6T (L250, V254 and H258) are colored orange. The S6T residue L353 is colored purple. Note that in the closed state residue L353 comes in close proximity to residue V254 in S4S5L. The “high impact” residues W248, L251 and V255 in S4S5L are highlighted in blue. (B) Similar representation as in panel A but now a top view on the interaction between S4S5L and S6T. Residues W248, L251, and V255 (blue side-chains) are directed towards the membrane interface whereas residues L250, V254 and H258 (in orange spacefill, except V254) are facing S6T. Note that the relative orientation of the blue and orange colored residues of S4S5L is the same in both the closed and open channel conformation. However, the side on S6T that contacts S4S5L is different in the closed versus the open state. Apparently L353 rotates towards the cytoplasmic mouth of the pore and away from V254 in S4S5L when transitioning from the closed to the open channel conformation. (C) Viewed from the internal side using the same color coding as in panel A but now showing all four subunits. The “low impact” residues F256, I257 and R249 (displayed in gray with their solvent accessible surface in transparent) locate on the side of S4S5L that faces away from S6T and are apparently not making protein contacts in either the closed or open conformation.
|                | V1/2 (mV) | k (mV) | $\tau_{V1/2+60}$ (ms) | n  | $\tau_{V1/2-100}$ (ms) | n  | $\Delta G_0$  | $\Delta \Delta G_0$ |
|----------------|-----------|--------|------------------------|----|------------------------|----|---------------|---------------------|
| KCNQ1          | -0.2 ± 2.5| 16 ± 0.9| 21 ± 1.9               | 15 | 97 ± 15                | 15 | -0.01 ± 0.10  | -0.08 ± 0.17        |
| T247A          | -2.2 ± 3.3| 14 ± 1.1| 69 ± 8.5               | 5  | 156 ± 29               | 5  | -0.09 ± 0.14  | -0.08 ± 0.17        |
| W248A          | 5.9 ± 1.7 | 9.6 ± 1.6| 30 ± 5.5               | 6  | 57 ± 11                | 6  | 0.36 ± 0.12   | 0.37 ± 0.15         |
| R249A          | 4.1 ± 1.4 | 17.4 ± 0.9| 45 ± 5.9              | 4  | 95 ± 4                 | 4  | 0.14 ± 0.05   | 0.15 ± 0.10         |
| L250A          | NF        | NF     | NF                     | -  | NF                     | -  | -             | -                   |
| L251A          | 27 ± 2.3  | 9.2 ± 0.9| 150 ± 9               | 7  | 310 ± 24               | 7  | 1.72 ± 0.22   | 1.73 ± 0.23         |
| G252A          | -8.2 ± 3.3| 13 ± 0.9| 51 ± 8.4               | 6  | 150 ± 5.0              | 6  | -0.37 ± 0.15  | -0.36 ± 0.17        |
| G252W          | -14 ± 2.4 | 9.2 ± 1.0| 16 ± 1.3              | 4  | 93 ± 20                | 4  | -0.89 ± 0.18  | -0.88 ± 0.20        |
| S253A          | -0.4 ± 4.6| 17 ± 1.6| 20 ± 1.4              | 5  | 120 ± 10               | 5  | -0.01 ± 0.15  | 0.00 ± 0.18         |
| S253W          | 2.7 ± 3.2 | 17 ± 1.3| 32 ± 6.0              | 5  | 130 ± 30               | 5  | 0.09 ± 0.11   | 0.10 ± 0.14         |
| V254A          | 4.7 ± 2.7 | 14 ± 1.2| 51 ± 8.0              | 6  | 220 ± 28               | 6  | 0.18 ± 0.14   | 0.19 ± 0.17         |
| V254W          | NE        | NE     | NE                    | -  | NE                     | -  | -             | -                   |
| V255A          | 3.4 ± 3.1 | 15 ± 1.0| 54 ± 11               | 6  | 130 ± 18               | 6  | 0.13 ± 0.12   | 0.14 ± 0.15         |
| V255W          | 34 ± 1.4  | 11 ± 0.2| 150 ± 17*             | 4  | 100 ± 33               | 4  | 1.81 ± 0.08   | 1.82 ± 0.12         |
| F256A          | 2.8 ± 3.5 | 12 ± 0.6| 38 ± 6                | 6  | 110 ± 11               | 6  | 0.22 ± 0.11   | 0.23 ± 0.14         |
| I257A          | -6.8 ± 3.0| 10 ± 0.6| 38 ± 5                | 5  | 81 ± 14                | 5  | -0.40 ± 0.17  | -0.39 ± 0.20        |
| H258A          | 38 ± 2.4  | 12 ± 1.2| 770 ± 150            | 5  | 339 ± 16               | 5  | 1.86 ± 0.22   | 1.87 ± 0.47         |
| R259A          | 32 ± 3.0  | 13 ± 2.5| 58 ± 5.8              | 5  | 95 ± 37                | 5  | 1.44 ± 0.30   | 1.45 ± 0.32         |
| Q260A          | 0.7 ± 3.0 | 12 ± 1.8| 38 ± 3.0             | 8  | 120 ± 0.6              | 8  | 0.03 ± 0.14   | 0.04 ± 0.17         |
|          | activation | deactivation |
|----------|------------|--------------|
|          | $V_{1/2}$ (mV) | k (mV) | $\tau_{(V_{1/2} +60)}$ (ms) | n | $\tau_{(V_{1/2} -100)}$ (ms) | n | $\Delta G_0$ | $\Delta G_0$ |
| KCNQ1    | -0.2 ± 2.5 | 16 ± 0.9 | 21 ± 1.9 | 15 | 97 ± 15 | 354 ± 69 | 8 | -0.01 ± 0.09 |
| V254A$^\S$ | 4.7 ± 2.7 | 14 ± 1.2 | 51 ± 8.0 | 6 | 220 ± 28 | 1000 ± 140 | 4 | 0.18 ± 0.14 | 0.19 ± 0.17 |
| V254L$^\S$ | -3.1 ± 4.9 | 18 ± 1.9 | 22 ± 2.7 | 6 | ND | ND | ND | -0.10 ± 0.16 | -0.09 ± 0.18 |
| V254E$^\S$ | 73 ± 5.0 | 17 ± 3.5 | 28 ± 1.3* | 5 | 150 ± 15** | 300 ± 25*** | 4 | 2.52 ± 0.54 | 2.53 ± 0.55 |
| V254K    | NE | NE | NE | NE | NE | NE | NE | NE |
| L353A$^\S$ | -1.0 ± 2.1 | 18 ± 2.0 | 28 ± 2.1 | 10 | 150 ± 12 | 570 ± 34 | 9 | -0.03 ± 0.07 | 0.04 ± 0.11 |
| L353E$^\S$ | -22 ± 3.0 | 17 ± 1.1 | 28 ± 6.6 | 5 | 170 ± 40 | 280 ± 78 | 4 | -0.76 ± 0.11 | -0.75 ± 0.15 |
| L353K$^\S$ | 2.7 ± 3.8 | 19 ± 2.1 | 10 ± 2.1 | 5 | 320 ± 50 | 1800 ± 170 | 7 | 0.08 ± 0.12 | 0.09 ± 0.15 |
| V254L+L353A | 24.7 ± 2.7 | 17 ± 0.9 | 204 ± 13 | 7 | 117 ± 12 | 336 ± 15 | 5 | 0.85 ± 0.10 | 0.86 ± 0.14 |
| V254K+L353E | NE | NE | NE | NE | NE | NE | NE | NE |
| V254E+L353K | NE | NE | NE | NE | NE | NE | NE | NE |
| L250K+L353E | 16.4 ± 1.3 | 22 ± 2.2 | 172 ± 10 | 6 | 64 ± 7.0 | 208 ± 22 | 6 | 0.43 ± 0.06 | 0.44 ± 0.11 |
| H258K+L353E | 13.3 ± 2.6 | 16.2 ± 1.0 | 109 ± 6 | 5 | 204 ± 24 | 2020 ± 240 | 5 | 0.48 ± 0.10 | 0.49 ± 0.13 |
| L250K    | NE | NE | NE | NE | NE | NE | NE | NE |
| H258K    | 30.1 ± 2.3 | 15.2 ± 1.2 | 237 ± 24* | 3 | 402 ± 60 | 1790 ± 90 | 4 | 1.16 ± 0.13 | 1.17 ± 0.16 |
Figure 1

A

WT KCNQ1

+70 mV

-40 mV

300 ms

300 pA

L251A

V255W

H258A

R259A

T247A

+80 mV

-40 mV

500 ms

100 pA

50 pA

250 pA

B

C

normalised current

KCNO1

L251A

V255W

H258A

R259A

T247A

normalised current

KCNO1

L251A

V255W

H258A

R259A

T247A

tau (ms)
Figure 2

A

+70 mV
-40 mV
300 ms
150 pA

V_m (mV)

W248A

+30 mV
+60 mV
tails WT

+110 mV
0 mV

500 ms
500 pA

B

Normalized current

KCNQ1
W248A

normalised current

C

Normalized current

normalised current

V_m (mV)

V_m (mV)
The S4-S5 linker of KCNQ1 channels forms a structural scaffold with the S6 segment controlling gate closure

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