



The KCNE tango – how KCNE1 interacts with Kv7.1

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The classical tango is a dance characterized by a 2/4 or 4/4 rhythm in which the partners dance in a coordinated way, allowing dynamic contact. There is a surprising similarity between the tango and how KCNE β -subunits “dance” to the fast rhythm of the cell with their partners from the Kv channel family. The five KCNE β -subunits interact with several members of the Kv channels, thereby modifying channel gating via the interaction of their single transmembrane-spanning segment, the extracellular amino terminus, and/or the intracellular carboxy terminus with the Kv α -subunit. Best studied is the molecular basis of interactions between KCNE1 and Kv7.1, which, together, supposedly form the native cardiac I_{Ks} channel. Here we review the current knowledge about functional and molecular interactions of KCNE1 with Kv7.1 and try to summarize and interpret the tango of the KCNEs.

Keywords: Kv channel, Kv7.1, β -subunit, KCNE1

INTRODUCTION

Voltage-gated potassium channels (Kv) are ubiquitously expressed in human tissues. They enable the rapid, selective movement of potassium ions through cellular membranes, thereby regulating physiological processes such as transmembrane ion passage and hormone secretion, vesicle cycling, and cell excitability. Kv channels display a huge diversity due to the large number of different α -subunits, alternative splicing, post-transcriptional modifications, and their ability to form heteromeric channels with other pore-forming α -subunits. To complicate the situation even more, the number of functionally different Kv channels in native tissues is further increased by interaction with regulatory β -subunits. These accessory subunits modify subcellular localization as well as biophysical properties of Kv channels such as gating kinetics, ion selectivity, and pharmacology. Among the best studied modulations of Kv channels by regulatory β -subunits is the effect of KCNE1 on Kv7.1.

Kv7.1-EXPRESSION AND PHYSIOLOGICAL FUNCTION

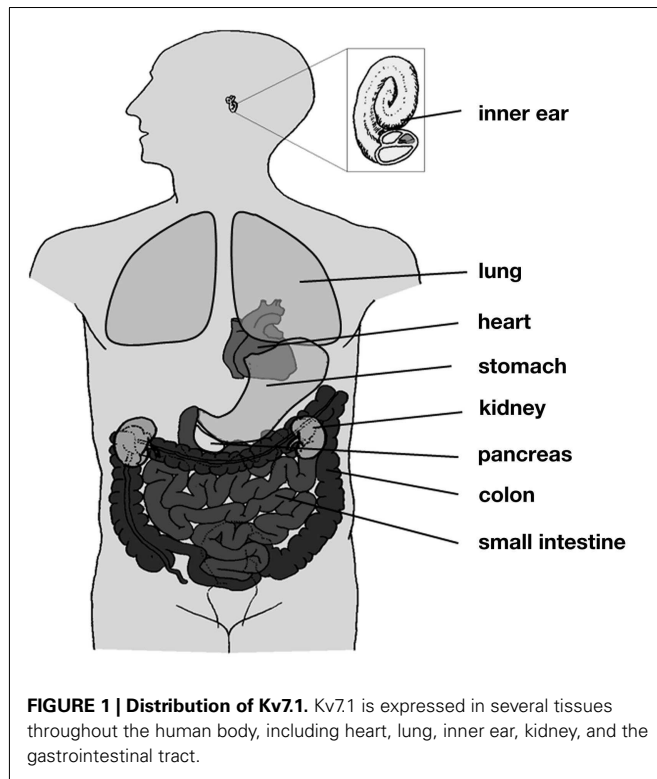
The *KCNQ1* gene was first identified by Wang et al. (1996b) in a linkage study of patients with long QT syndrome (LQTS1). Its gene product, Kv7.1 (also termed KvLQT1 or KCNQ1), is a voltage-gated potassium channel α -subunit, and its expression was detected in several mammalian tissues, including heart, epithelia, and smooth muscle (Figure 1; Table A1 in Appendix). Kv7.1 can assemble with different members of the KCNE family of regulatory β -subunits to fulfill a variety of physiological functions.

In the heart, Kv7.1 is involved in the termination of the cardiac action potential. The repolarizing potassium current, I_{Kr} , consists of two major components, the rapid delayed rectifier potassium current I_{Kr} and the slow delayed rectifier potassium current I_{Ks} (Sanguinetti and Jurkiewicz, 1990). Kv7.1 coassembles with the KCNE1 β -subunit to form the channel complex that mediates I_{Ks}

(Barhanin et al., 1996; Sanguinetti et al., 1996). Although KCNE1 is the major accessory subunit assembling with Kv7.1 in the heart, other subunits of the KCNE family might be present (Bendahhou et al., 2005), serving as additional regulators of I_{Ks} (Wu et al., 2006). The significance of Kv7.1 and its accessory β -subunits for maintaining normal rhythmicity is further emphasized by the numerous *KCNQ1* and *KCNE1* mutations associated with cardiac arrhythmias (<http://www.fsm.it/cardmoc/>). Most of these mutations lead to loss of channel function causing LQTS, a disorder predisposing affected individuals to *torsade de pointes* arrhythmia and cardiac sudden death.

Besides its cardiac function, several lines of evidence suggest an important role of Kv7.1 and its accessory β -subunit KCNE1 in the hearing process. In patients suffering from Jervell and Lange-Nielsen syndrome – the recessive form of inherited LQTS – cardiac arrhythmia is accompanied by profound bilateral deafness. Mutations in both *KCNQ1* and *KCNE1* genes have been reported to cause this disorder (Jervell and Lange-Nielsen, 1957; Neyroud et al., 1997; Schulze-Bahr et al., 1997). In addition, targeted disruption of the *KCNQ1* gene in mice leads to deafness caused by morphological abnormalities of the inner ear (Lee et al., 2000; Casimiro et al., 2001). Expression of Kv7.1 and KCNE1 has been detected in the marginal cells of the *stria vascularis* of the cochlea and the vestibular dark cells (Neyroud et al., 1997; Nicolas et al., 2001; Knipper et al., 2006; Hur et al., 2007). Both cell types are involved in the generation of the potassium-rich endolymph, and Kv7.1/KCNE1 channels have been suggested to be key mediators of this K^+ secretion (Marcus and Shen, 1994; Shen et al., 1995; Wangemann, 1995; Wangemann et al., 1995; Sunose et al., 1997).

In addition to the inner ear epithelium, Kv7.1 has been detected in a variety of other epithelial cell types, where it participates in secretory transduction. In the kidney, Kv7.1/KCNE1 channels seem to be located in the proximal tubule of the nephron



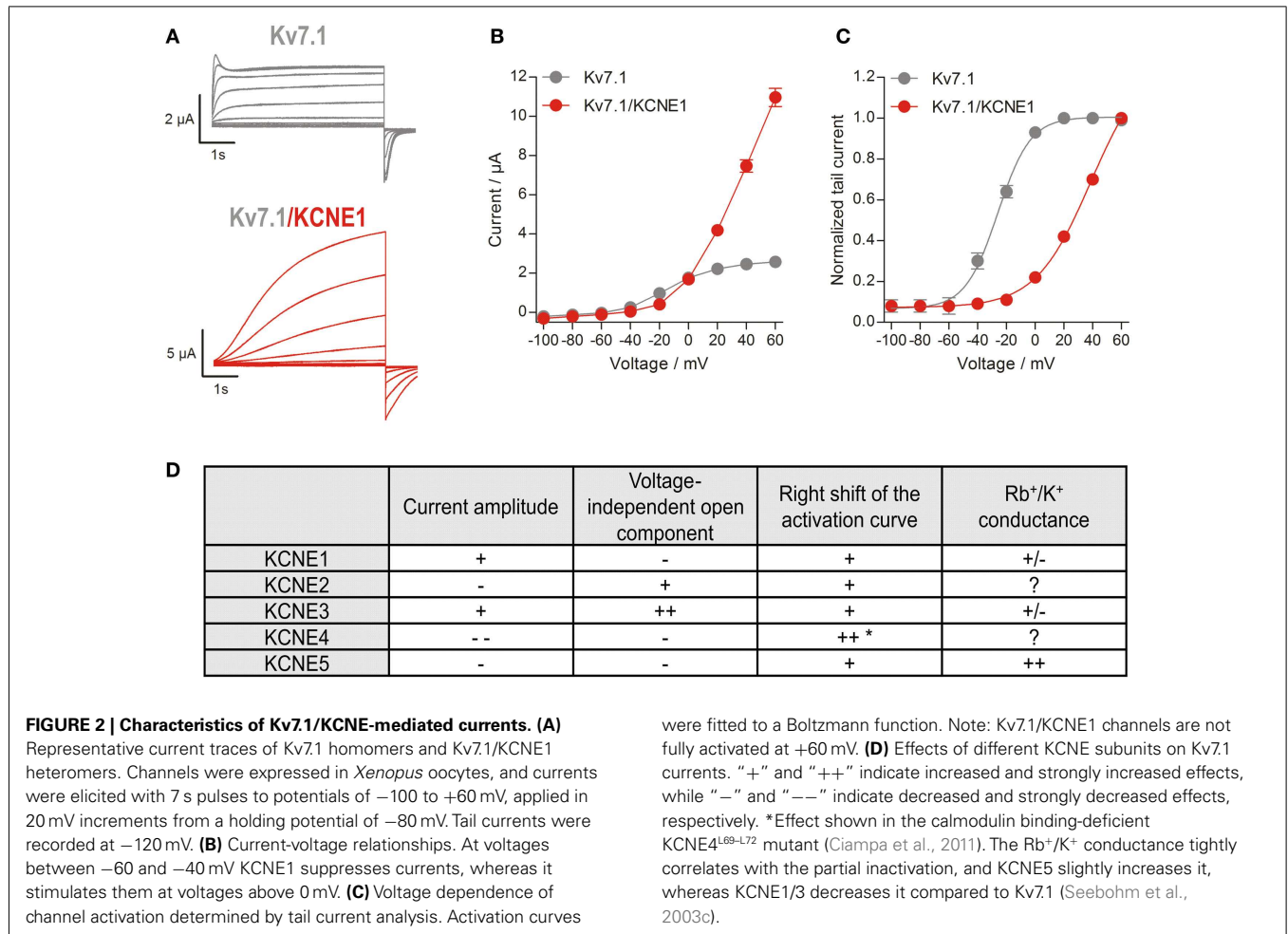
(Sugimoto et al., 1990; Vallon et al., 2001), conducting a K^+ current to counterbalance membrane depolarization induced by electrogenic Na^+ -coupled transport of glucose or amino acids (Vallon et al., 2001, 2005). The relevance of Kv7.1/KCNE1 channels for renal function is further underlined by the observation that KCNE1 knockout mice suffer from hypokalemia, urinary and fecal salt wasting, and volume depletion (Arrighi et al., 2001; Warth and Barhanin, 2002). Kv7.1 expression has also been detected in the small intestine and the colon (Schroeder et al., 2000; Dedek and Waldegger, 2001; Demolombe et al., 2001; Kunzelmann et al., 2001; Horikawa et al., 2005). In colonic crypt cells Kv7.1 is believed to assemble with another accessory β -subunit, KCNE3, and to mediate a K^+ conductance that provides the driving force for chloride secretion (Schroeder et al., 2000; Kunzelmann et al., 2001). Two further examples of Kv7.1 expression and function in chloride-secreting tissues are pancreatic acinar cells and airway epithelium (Kim and Greger, 1999; Kottgen et al., 1999; Mall et al., 2000; Demolombe et al., 2001; Grahammer et al., 2001b; Lee et al., 2004). In parietal cells of the stomach Kv7.1 coassembles with KCNE2 and participates in gastric acid secretion (Dedek and Waldegger, 2001; Demolombe et al., 2001; Grahammer et al., 2001a; Heitzmann et al., 2004). In KCNQ1 knockout mice gastric hyperplasia and profound hypochlorhydria have been observed, indicating the importance of Kv7.1 in normal stomach development and function (Lee et al., 2000). Kv7.1 expression has also been detected in the human thyroid gland, and it has been shown that mice lacking functional Kv7.1 develop hypothyroidism (Frohlich et al., 2011). Recently, Kv7.1 channels have been shown to relax systemic and pulmonary arteries upon pharmacological activation (Chadha et al., 2012).

REGULATION OF Kv7.1 BY ACCESSORY β -SUBUNITS OF THE KCNE GENE FAMILY

All five members of the KCNE family of regulatory β -subunits can functionally coassemble with Kv7.1 to subunit-specifically change its current characteristics (for an excellent review about KCNEs, see McCrossan and Abbott, 2004). The founding member KCNE1 (previously termed MinK or I_{sK}) was identified in 1988 (Takumi et al., 1988). As its expression in *Xenopus* oocytes produced slowly activating potassium currents resembling the cardiac I_{Ks} , KCNE1 was originally believed to be the ion channel α -subunit forming the I_{Ks} channel. However, it is now well established that KCNE1 cannot form functional K^+ channels alone, but can serve as a regulatory β -subunit for several voltage-gated cation channels, including Kv7.1 (Barhanin et al., 1996; Sanguinetti et al., 1996). The I_{Ks} -like potassium currents observed in oocytes upon heterologous expression of KCNE1 were actually caused by coassembly with endogenous Kv7.1. So far, four other members of the KCNE family, KCNE2–KCNE5 (also termed MiRP1–4) have been identified (Abbott et al., 1999; Piccini et al., 1999), and their influence on Kv7.1 channels has been studied extensively in heterologous expression systems. Kv7.1 expressed alone forms a classical delayed rectifier potassium-selective channel with fast activation, delayed partial inactivation, and relatively slow deactivation (Pusch et al., 1998; Tristani-Firouzi and Sanguinetti, 1998). The presence of KCNE1 drastically modifies Kv7.1 activity by increasing unitary conductance as well as macroscopic currents, slowing activation, right-shifting voltage dependence of activation, suppressing currents at low activating voltages, suppressing partial inactivation, increasing Q_{10} -value, and modulating pharmacology (Figures 2A–C; Barhanin et al., 1996; Sanguinetti et al., 1996; Pusch, 1998; Pusch et al., 1998; Sesti and Goldstein, 1998; Tristani-Firouzi and Sanguinetti, 1998; Lerche et al., 2000; Seeböhm et al., 2001a,b,c, 2003a,b,c, 2005; Morokuma et al., 2008). Coassembly of Kv7.1 and KCNE2 gives rise to currents with decreased amplitude, instantaneous activation, rapid partial deactivation, and a linear current-voltage relationship (Tinel et al., 2000). KCNE3 also converts Kv7.1 to a channel with nearly instantaneous activation and a linear current-voltage relationship (Schroeder et al., 2000; Seeböhm et al., 2003c), but in contrast to Kv7.1/KCNE2, complexes containing KCNE3 show some time dependency of gating at positive potentials and increased current densities (Schroeder et al., 2000; Melman et al., 2001; Mazhari et al., 2002). KCNE4 completely suppresses Kv7.1 currents at physiologically relevant membrane potentials (Grunnet et al., 2002) and KCNE5 shifts the voltage dependence of Kv7.1 activation to more positive potentials toward an activation threshold of about +40 mV (Figure 2D; Angelo et al., 2002; Seeböhm et al., 2003c).

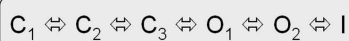
GATING OF Kv7.1 AND ITS MODULATION BY KCNE1

The biophysical properties of voltage-gated Kv7.1 channels change dramatically when they coassemble with KCNEs. Homotetrameric Kv7.1 channels activate with relatively fast kinetics with $\tau_{activation}$ by about 80–100 ms at 60 mV (Figure 2; Pusch et al., 1998; Tristani-Firouzi and Sanguinetti, 1998). The channels undergo partial inactivation, with about 60% of the channels being inactivated at 60 mV in steady-state (Figure 2). The kinetic behavior of



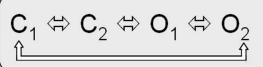
Kv7.1 can be approximated by a linear gating scheme of the form (Pusch et al., 1998; Tristani-Firouzi and Sanguinetti, 1998):

model 1:



In this model $C_1 \rightleftharpoons C_2$, $C_2 \rightleftharpoons C_3$, and $O_1 \rightleftharpoons O_2$ are the voltage-dependent gating steps. Recently, an alternative circular gating scheme was proposed by Ma et al. (2011).

model 2:

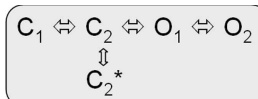


This novel gating scheme accounts for an open fraction that is determined by allosteric coupling of residues in the S4–S5 linker/pore domain (PD). $C_1 \rightleftharpoons C_2$ and $O_1 \rightleftharpoons O_2$ are the voltage-dependent gating steps.

Kv7.1/KCNE1 heteromeric channels activate far more slowly and at more positive potentials than homomeric Kv7.1 channels, do not partially inactivate, and have an increased single-channel conductance compared to homomeric Kv7.1 recorded in standard

extracellular solutions (Barhanin et al., 1996; Sanguinetti et al., 1996; Pusch, 1998; Pusch et al., 1998; Tristani-Firouzi and Sanguinetti, 1998). It is believed that Kv7.1/KCNE1 channel currents resemble the cardiac repolarizing, slowly activating potassium current I_{Ks} and that LQTS1/5 mutations might directly or allosterically modify interactions of Kv7.1 and the β -subunit KCNE1 (e.g., Schmitt et al., 2000; Seeböhm et al., 2001c; Osteen et al., 2010; Wang et al., 2011b). Cui et al. (1994) proposed two linear-branched gating schemes for Kv7.1/KCNE1 heteromers, which depend on varying KCNE1 amounts coexpressed with Kv7.1 and imply interactions among individual channel proteins during activation. Yet Tzounopoulos et al. reported on a crossover gating phenomenon after prepulsing to different voltages that cannot be explained by a classical Cole–Moore shift in a linear gating model of Kv7.1/KCNE1 channels and presented results that argue for a circular gating model similar to model 2 (Cole and Moore, 1960; Tzounopoulos et al., 1998). However, a branched model can account for this crossover gating phenomenon after prepulsing to different voltages as well (Strutz-Seeböhm et al., 2011):

model 3:



Further gating schemes have been proposed to describe Kv7.1/KCNE1 gating (Silva and Rudy, 2005, 2010; Osteen et al., 2010; Ghosh et al., 2011). In summary, the gating behaviors of Kv7.1 and Kv7.1/KCNE1 are relatively complex, and simple, linear gating models reach too short to approximate a realistic mathematical description.

STOICHIOMETRY OF THE Kv7.1/KCNE1 COMPLEX

After the discovery that the channel complex mediating the cardiac I_{Ks} is composed of Kv7.1 α -subunits and KCNE1 β -subunits (Barhanin et al., 1996; Sanguinetti et al., 1996), detailed investigations on the nature of this association started. Like all members of the Kv channel family, Kv7.1 α -subunits assemble into tetramers to form functional channels. Each α -subunit consists of six transmembrane segments, S1–S6, flanked by intracellular amino- and carboxy-terminal domains. The central pore domain (PD) of the channel, which contains the ion conduction pathway and the activation gate, is formed by segments S5 and S6 and the pore loop between them. The pore loop carries the K⁺ channel signature sequence TTIGYGD allowing rapid ion conduction and potassium selectivity. The PD is surrounded and controlled by four voltage-sensing domains (VSDs) formed by segments S1–S4 from each subunit (Figures 3A,B). S4 contains several positively charged amino acids and is a key element of voltage sensing. Further charged residues in S1–S3 may contribute additional charge to allow for voltage sensing. Tetrameric assembly of Kv7.1 α -subunits is mediated via their C-terminal domains (Schmitt et al., 2000; Schwake et al., 2003). Although Kv7.1 homotetramers are functional, the presence of the accessory β -subunit KCNE1 is needed to produce currents with characteristics of native I_{Ks} (Figure 2A). KCNE1 is a small, integral membrane protein with a single transmembrane-spanning segment flanked by an extracellular N-terminal and an intracellular C-terminal domain (Figure 3A). The number of KCNE1 β -subunits recruited by tetrameric Kv7.1 channels has been a matter of extensive debate (Figure 3B). Previous studies have either suggested a fixed stoichiometry of four Kv7.1 subunits and two KCNE1 subunits (Wang and Goldstein, 1995; Chen et al., 2003a; Kang et al., 2008; Morin and Kobertz,

2008) or a variable stoichiometry with up to four KCNE1 subunits assembling with a Kv7.1 tetramer (Cui et al., 1994; Wang et al., 1998, 2011a; Morokuma et al., 2008; Nakajo et al., 2010; Zheng et al., 2010; Strutz-Seebohm et al., 2011). A summary of the data obtained during the last two decades is provided in Table 1. The results reported leave the possibility open that KCNE1 subunits coassemble with Kv7.1 in different modes allowing up to four KCNE1 subunits to approach the Kv7.1 tetramer but permitting only formation of 4 Kv7.1 : 2 KCNE1 assemblies in specific regions of the heteromeric channels. Prerequisites for such an interaction would be variable and dynamic interactions. Indeed, several studies suggest interactions of Kv7.1 with KCNE1 at different protein regions in the various channel states (summarized in Table 2). However, further studies are required to support such a hypothesis.

STRUCTURAL BASIS OF Kv7.1/KCNE1 INTERACTION

The structural basis of the Kv7.1/KCNE1 interaction has been studied extensively over the last 20 years. Experimental data obtained from deletion analysis, chimeric approaches, and site-directed mutagenesis identified regions in both Kv7.1 and KCNE1 that might be crucial for association and modulation (summarized in Table 2). Based on these data it has been suggested that the transmembrane segment and the C-terminal domain of KCNE1 mediate its ability to modulate Kv7.1 (Takumi et al., 1991; Tapper and George, 2000). Molecular key elements of KCNE1 function seem to be three glycines, several bulky, aromatic phenylalanines, and the threonine 58 located at the center of the KCNE1 transmembrane segment (Melman et al., 2001, 2002; Strutz-Seebohm et al., 2011). A comparison with other KCNE subunits underscores the importance of the transmembrane segment for KCNE function: interactions of Kv7.1 with KCNE1 and Kv7.1 with KCNE3 show similar chemical modification rates within the transmembrane region (Rocheleau and Kobertz, 2008), and the KCNE4 transmembrane segment modulates voltage dependence of Kv7.1 activation (Ciampa et al., 2011). A recent study indicates that different KCNE proteins contact different regions in Kv7.1 all located within the transmembrane segments (Nakajo et al., 2011). In segment S6 of Kv7.1, a three amino acid motif (S338, F339,

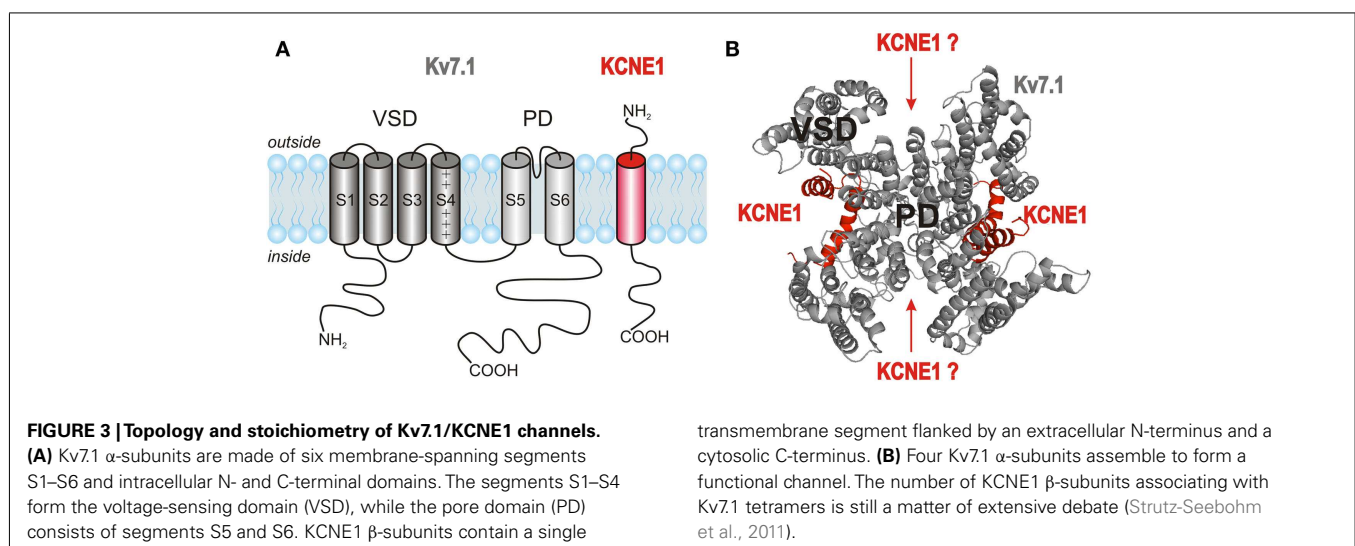


Table 1 | Stoichiometry of Kv7.1/KCNE1 channels.

Experimental evidence leading to conclusion of fixed stoichiometry (4:2)	Reference
Suppression of current induced by coexpression of wildtype and mutant KCNE1 indicates 4:2 stoichiometry	Wang and Goldstein (1995)
Kv7.1/Kv7.1/KCNE1 fusion proteins and naturally assembled Kv7.1/KCNE1 channels show similar characteristics of CTX inhibition; quantification of Kv7.1 and KCNE1 subunits using ³ H-CTX and an antibody indicates a 4:2 stoichiometry	Chen et al. (2003a)
Chemical subunit counting experiments indicate that association of two KCNE1 subunits with the Kv7.1 tetramer is sufficient to induce KCNE1-typical modulation of channel properties	Morin and Kobertz (2008)
Computational model of Kv7.1/KCNE1 channels indicates that binding of more than two KCNE1 subunits to the Kv7.1 tetramer might be sterically hindered	Kang et al. (2008)
Experimental evidence leading to conclusion of variable stoichiometry	Reference
Current amplitude, activation kinetics, and voltage dependence of Kv7.1/KCNE1 channels vary with the amount of KCNE1	Cui et al. (1994)
Both Kv7.1/KCNE1 fusion proteins and Kv7.1–Kv7.1 with additional KCNE1 produce currents with activation kinetics and voltage dependence similar to naturally assembled Kv7.1/KCNE1 channels	Wang et al. (1998)
Voltage dependence of activation of Kv7.1/KCNE1 channels varies with the amount of KCNE1	Morokuma et al. (2008)
Single-molecule fluorescent bleaching studies indicate that up to four KCNE1 subunits associate with the Kv7.1 tetramer depending on the relative densities of the two subunits	Nakajo et al. (2010)
Effects of free KCNE1 C-terminals on voltage dependence of activation of Kv7.1 and Kv7.1/KCNE1 channels are complex, indicating multiple stoichiometries or saturation of possible binding sites	Zheng et al. (2010)
Overexpression of KCNE1 markedly changed activation kinetics and voltage dependence of native <i>I_{Ks}</i> , indicating assembly of additional KCNE1 subunits with endogenous channels	Wang et al. (2011a)
Modeling of transmembrane domain suggests that variable stoichiometry seems possible in this region	Strutz-Seebohm et al. (2011)

F340) has been identified that might constitute a site of specific interaction with KCNE1, especially with its residue T58 (Melman et al., 2004; Panaghie et al., 2006; Strutz-Seebohm et al., 2011). Further residues in the pore region of Kv7.1 (F270, G272, and L273 in S5, V307, V310, and T311 in the lower pore helix, and V324 and V334 in the upper S6) are crucial for an accurate modulation by KCNE1 (Seebohm et al., 2001c, 2003c; Nakajo et al., 2011).

Based on experimental evidence, localizations of KCNE1 in the channel complex have been suggested. A series of observations obtained from cysteine scanning mutagenesis combined with chemical modifications (Cd²⁺ coordination, MTS reagent binding, and spontaneous disulfide formation) indicate that KCNE1 lies in close proximity to both the PD and the VSD of the Kv7.1 tetramer (summarized in **Table 2**). First interpretations suggested that KCNE1 lines the conduction pathway (Wang et al., 1996a; Tai and Goldstein, 1998). However, subsequent studies indicate that the KCNE1 transmembrane segment is located outside the PD and interacts with the outer S5 and S6 segments of Kv7.1 (Lerche et al., 2000; Kurokawa et al., 2001; Tapper and George, 2001; Chung et al., 2009; Strutz-Seebohm et al., 2011). Its extracellular flank seems to face the extracellular ends of segments S1 and S6 of Kv7.1, with the residues interacting with segment S1 varying dependent on the specific channel state (Xu et al., 2008; Chung et al., 2009). In addition to segment S1 of the VSD and S5/S6 of the PD, the transmembrane domain of KCNE1 gets in close contact with the primary cationic voltage sensor S4 (Nakajo and Kubo, 2007; Shamgar et al., 2008; Silva et al., 2009; Strutz-Seebohm et al., 2011). A possible interaction of KCNE1 with the VSD of Kv7.1 is further underscored by the fact that the abnormal phenotypes of several disease-causing mutations in S1, S4, or the S4–S5 linker of Kv7.1 *in vitro* only manifest in the presence of KCNE1 (summarized in

Table 2; Franqueza et al., 1999; Chouabe et al., 2000; Chen et al., 2003c; Chan et al., 2012). The cytoplasmic portion directly following the transmembrane segment of KCNE1 seems to interact with the gating machinery of Kv7.1 (Lvov et al., 2010). A hallmark of all Kv7 channels is their sensitivity to muscarinic signaling, which gave them the name M-channels. The Kv7.1 membrane-proximal region may link membrane lipid metabolism of phosphoinositide PI(4,5)P₂ to the gating machinery to allow for integration of muscarinic signaling into gating alterations in Kv7.1 (Ikeda and Kammermeier, 2002; Loussouarn et al., 2003). The lower S6 is expected to be in close proximity to allow for electrostatic interaction with the PI(4,5)P₂ head groups, and PI(4,5)P₂ binding sites have been reported to be positioned close to the lower S6-helices bundle in the PD of Kv7.1 (Loussouarn et al., 2003; Thomas et al., 2011). However, it may be mentioned that further PI(4,5)P₂ binding sites may exist in the TM domain. KCNE1 residues R⁶⁷SKKLEH⁷³ in the vicinity of the inner membrane leaflet increase the PI(4,5)P₂ sensitivity of Kv7.1 by a factor of 100, possibly by electrostatic interaction with PI(4,5)P₂ head groups and/or Kv7.1 residues interacting with PI(4,5)P₂ (Li et al., 2011). Thus, PI(4,5)P₂ may function as a molecular coupler of Kv7.1 and KCNE1 to modulate gating in complex ways.

Recent structural modeling approaches generated 3D models of the transmembrane region of Kv7.1/KCNE1 that are in large agreement with the various experimental data. Specifically, homology modeling of the Kv7.1 S1–S6 region and *Rosetta*-based docking of the KCNE1 transmembrane domain produced an open and a closed state model of the heteromeric channel complex (Smith et al., 2007; Kang et al., 2008). Very recently, homology modeling of Kv7.1 with manual docking of the KCNE1 transmembrane domain was used to generate a pre-open closed state model of

Table 2 | Structural basis of Kv7.1/KCNE1 interaction.

Approach	Conclusion	Reference
Deletion analysis, chimeric approach, and/or site-directed mutagenesis	TM segment and cytoplasmic portion immediately following TM segment of KCNE1 mediate KCNE1 function	Takumi et al. (1991)
	TM segment and C-terminal domain of KCNE1 mediate KCNE1 function	Tapper and George (2000)
	Residues 57–59 of KCNE1 are important for KCNE1 function (“activation triplet”)	Melman et al. (2001)
	Residue L273 of Kv7.1 is important for normal modulation by KCNE1	Seeböhm et al. (2001c)
	Residue T58 is a key element of KCNE1 function	Melman et al. (2002)
	Requirements to interact with KCNE1 are located in regions C-terminal to S5;	Melman et al. (2004)
	Residues S338, F339, and F340 in S6 are important for normal modulation by KCNE1	
	Residues S338, F339, and F340 in S6 are possible interaction sites of KCNE1	Panaghie et al. (2006)
	KCNE1 C-terminus is crucial for channel assembly, open state destabilization, kinetics of deactivation	Chen et al. (2009)
	Amino acids important for normal modulation by KCNE1 are located in S5 and S6 (G272, V324, V334) of Kv7.1	Nakajo et al. (2011)
Cysteine scanning mutagenesis combined with chemical modifications (Cd ²⁺ coordination, MTS reagent binding, and spontaneous disulfide formation)	TM segment of KCNE1 lines the conduction pathway	Wang et al. (1996a)
	TM segment of KCNE1 lines the conduction pathway	Tai and Goldstein (1998)
	KCNE1 is located outside the conduction pathway	Kurokawa et al. (2001)
	KCNE1 is located outside the conduction pathway but in very close proximity to S6 of Kv7.1	Tapper and George (2001)
	E44 in KCNE1 is close to A226 in S4 of Kv7.1 in the open state; KCNE1 is close to or possibly interacts with the VSD	Nakajo and Kubo (2007)
	KCNE1 makes state-dependent contact with S1 of Kv7.1; KCNE1 is in close proximity to the VSD	Xu et al. (2008)
Proof of direct physical interaction or close proximity (e.g., co-immunoprecipitation, FRET)	KCNE1 is located close to S1 and S4 of two adjacent VSDs	Shamgar et al. (2008)
	C-terminal region directly following the KCNE1 TM segment interacts with activation gate of Kv7.1	Lvov et al. (2010)
	C-terminus of KCNE1 directly interacts with the pore region of Kv7.1	Romey et al. (1997)
	C-termini of Kv7.1 and KCNE1 move close to each other during channel activation; distal C-terminus of KCNE1 interacts with dimeric coiled coil helix C of Kv7.1	Haitin et al. (2009)
Analysis of disease-causing mutations	Physical interaction between C-termini of Kv7.1 and KCNE1; portion of A-helix and its linker to S6 bind KCNE1	Zheng et al. (2010)
	Functional interaction of KCNE1 with S4/S4–S5 linker of Kv7.1	Franqueza et al. (1999)
	Functional interaction of KCNE1 with S4/S4–S5 linker of Kv7.1	Chouabe et al. (2000)
Computational model	Functional interaction of KCNE1 with S1 of Kv7.1	Chan et al. (2012)
	KCNE1 is located in a cleft between the pore domain and the VSD of Kv7.1	Kang et al. (2008)
	KCNE1 binds to the outer face of the Kv7.1 channel pore, KCNE1 is located in a cleft between pore domain and VSD of Kv7.1	Strutz-Seeböhm et al. (2011)

the Kv7.1/KCNE1 channel (Strutz-Seeböhm et al., 2011). All three models are relatively stable in molecular dynamics simulations, which suggests that they are close to native states.

The C-termini of Kv7.1 and KCNE1 are in close proximity and physically interact, as indicated by FRET measurements and co-immunoprecipitation, yet the structural basis of this interaction is unknown (Haitin et al., 2009; Zheng et al., 2010). The cytosolic domain of Kv7.1/KCNE1 channels represents a multi-modular structure serving multiple functions. It contains several amphipathic α -helices. Structural prediction algorithms place two α -helices, A and B, to the proximal C-terminus of Kv7.1 (Haitin and Attali, 2008). These α -helices may contain functionally relevant calmodulin interaction motifs. The residues Kv7.1^{586–618}

form α -helix D that contain a leucine-zipper and a coiled coil fold (Kanki et al., 2004). This parallel, four-stranded coiled coil allows for specific tetrameric assembly of Kv7.1 channel subunits (Schmitt et al., 2000; Schwake et al., 2003; Nakajo and Kubo, 2008). Recently, Xu and Minor (2009) presented a high-resolution X-ray crystal structure (1.7 Å resolution) of Kv7.1^{583–611} confirming the predictions of the coiled coil assembly specificity domain. Interestingly, helix D is supposedly two helical turns longer in this crystal structure than helices D of the closely related Kv7.2–5 channels, which allows for specific homomeric assembly with other Kv7.1 subunits but not for heteromeric assemblies with other family members (Haitin and Attali, 2008; Xu and Minor, 2009). The S6 transmembrane segment and the

A-domain are linked by the region Kv7.1^{350–582}, which according to *in silico* prediction assumes an apo-chloroperoxidase fold and can thus be modeled using the structural coordinates of apo-chloroperoxidase (pdb: 1VNS; Macedo-Ribeiro et al., 1999). However, these predictions appear somewhat preliminary, and several assumptions have to be made to incorporate the modeled region into the rest of the Kv7.1–3D model. Further, how exactly the proposed helices A/B and linker region containing the calmodulin binding site can be integrated into this fold is speculative. The structural folds of the N-terminal region Kv7.1^{1–115} and the most distal residues Kv7.1^{612–676} remain completely unknown.

PI(4,5)P₂ modulates function of Kv7.1/KCNE1 channels, and functional PI(4,5)P₂ interaction sites were reported not only for the lower S6 segment but also for the C-terminus, where a cluster of basic amino acids may form a PI(4,5)P₂ interaction site allowing for electrostatic interactions with the PI(4,5)P₂ head groups (Loussouarn et al., 2003; Zhang et al., 2003; Delmas and Brown, 2005). This PI(4,5)P₂ interaction site may functionally and physically overlap with the calmodulin binding sites in the inter helix A/B linker region (Hernandez et al., 2008). The Kv7.1–calmodulin interaction can be modulated by coassembly with KCNE4. This modulation is dependent on a juxtamembrane tetra-leucine motif (L69–L72) in KCNE4. However, deletion of this motif leaves the kinetic effects on Kv7.1 mediated by the KCNE4 transmembrane domain intact (Ciampa et al., 2011). Possibly, PI(4,5)P₂ and calmodulin “glue” these C-terminal regions to the transmembrane proximal regions mentioned above and allow for modulation of gating at the activation gate and the VSD. Clearly, further evidence is needed to prove this hypothesis (Li et al., 2011).

The C-termini of Kv7.1/KCNE1 channels physically interact with several proteins like the AKAP yotiao, epidermal growth factor receptor kinase, and NEDD4.2 (Marx et al., 2002; Kanki et al., 2004; Kurokawa et al., 2004; Jespersen et al., 2007; Dong et al., 2010). The protein kinase A phosphorylates Ser27 in the N-terminus of Kv7.1 and modulates Kv7.1/KCNE1 function (Marx et al., 2002). This modulation critically depends on the presence of KCNE1 (Marx et al., 2002; Kurokawa et al., 2004). This observation suggests that the Kv7.1 N-terminus contributes to Kv7.1/KCNE1 subunit interaction. The binding sites for these proteins have to be located at the surface of the C-terminal structure to be accessible for interaction. This information will be of help for further structural modeling. A 3D model of the Kv7.1/KCNE1 channel satisfying most of the aforementioned hypotheses is shown in **Figure 4**.

TOWARD A DYNAMIC VIEW ON STRUCTURAL REARRANGEMENTS DURING Kv7.1/KCNE1 GATING

In the last 14 years a wealth of potassium channel crystal structures have been reported. Although central structural elements in Kv7.1 clearly differ from classical Shaker-type Kv channels, the available crystal structures are well-suited templates to model the Kv7.1 S1–S6 region in different states (Seeböhm et al., 2006; Silva et al., 2009). Direct interactions of the transmembrane segments of Kv7.1 with KCNE1 have been proposed, but the specific amino acid interactions remain controversial (summarized in **Table 2**). CD spectra

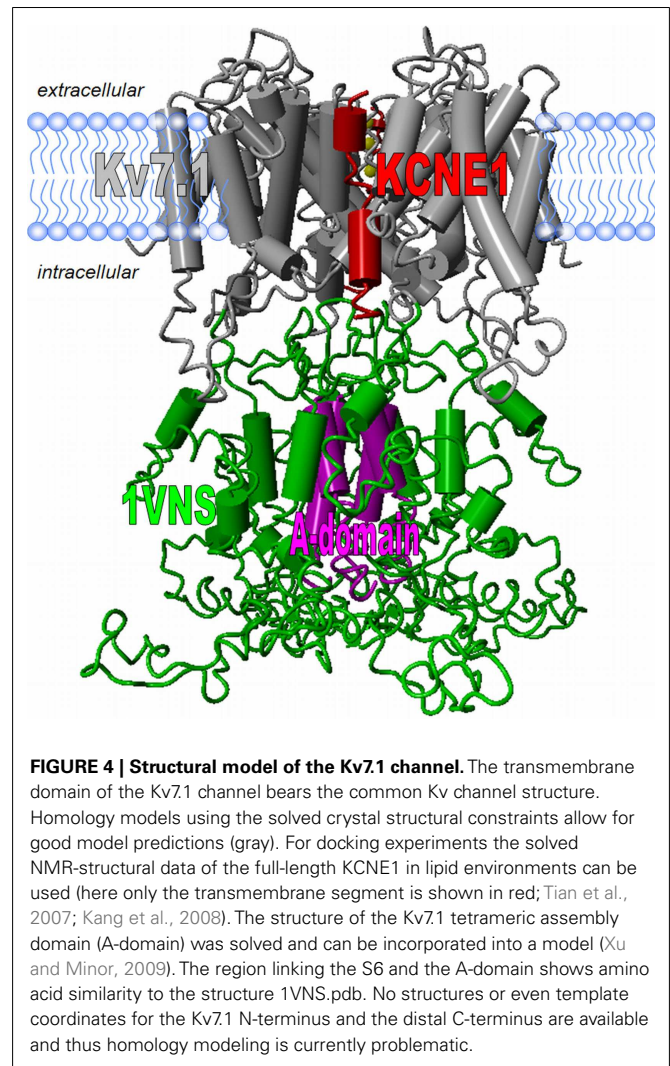


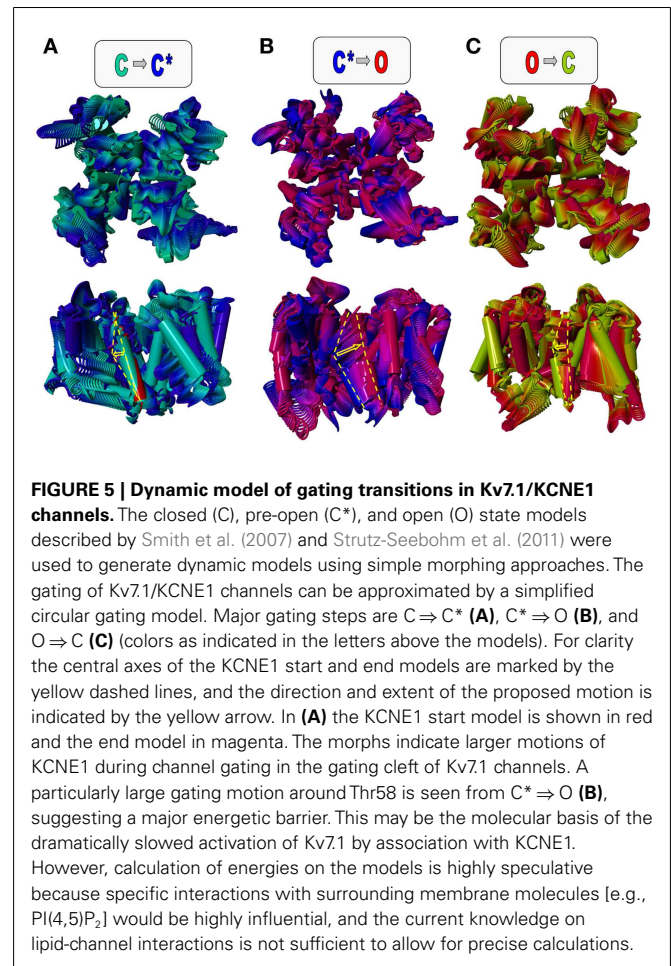
FIGURE 4 | Structural model of the Kv7.1 channel. The transmembrane domain of the Kv7.1 channel bears the common Kv channel structure. Homology models using the solved crystal structural constraints allow for good model predictions (gray). For docking experiments the solved NMR-structural data of the full-length KCNE1 in lipid environments can be used (here only the transmembrane segment is shown in red; Tian et al., 2007; Kang et al., 2008). The structure of the Kv7.1 tetrameric assembly domain (A-domain) was solved and can be incorporated into a model (Xu and Minor, 2009). The region linking the S6 and the A-domain shows amino acid similarity to the structure 1VNS.pdb. No structures or even template coordinates for the Kv7.1 N-terminus and the distal C-terminus are available and thus homology modeling is currently problematic.

in a membrane-like environment and the NMR solution structure of KCNE1 transmembrane peptides show that this peptide adopts an α -helical structure (Aggeli et al., 1998; Strutz-Seeböhm et al., 2011). Recently, two NMR structures of full-length KCNE1 confirmed that the transmembrane segment of KCNE1 folds an α -helix (Tian et al., 2007; Kang et al., 2008). An open and a closed state model of Kv7.1/KCNE1 channels were proposed based on the NMR-structural coordinates and *Rosetta*-dockings of KCNE1 to Kv7.1 that suggest binding of KCNE1 to the so-called “gain-of-function cleft”, a space formed between two adjacent voltage sensor domains and the outer PD (Smith et al., 2007; Kang et al., 2008).

Localized to the center of the α -helical KCNE1 transmembrane segment are the residues G⁵²FFGFFTLG⁶⁰. As predicted, this α -helical transmembrane domain allows for flexibility at glycine residues G52, G55, and G60 (Tian et al., 2007; Kang et al., 2008; Strutz-Seeböhm et al., 2011). Key to functional modulation of Kv7.1 by KCNE1 is the residue T58 (Melman et al., 2002). The surrounding phenylalanine residues generate a bulky aromatic cuff. The combination of these structural elements may enable formation of a stable hydrogen bond with residue S338 in Kv7.1 and

diverse van der Waals interactions with residues in S4 and the S4–S5 linker region as well as S5/S6 amino acids F270, F339, and F340 to stabilize a pre-open closed state. The combination of the Kv7.1/KCNE1 models, the closed state, the pre-open closed state and the open state model are in good agreement with published results of several experimental studies (Smith et al., 2007; Kang et al., 2008; Strutz-Seebohm et al., 2011). The modeled transmembrane domain structures can be used to assess a molecular view on the slow activation of I_{Ks} (Figure 5). The three states closed, pre-open, and open can be used for morphing calculations (Echols et al., 2003). The basic idea is that the channel undergoes conformational transitions from closed (C) to pre-open (C^*), from pre-open (C^*) to open (O), and from open (O) to closed (C) states. These seem to represent the major steps in the Kv7.1 and Kv7.1/KCNE1 gating (Silva et al., 2009). The position of the voltage sensor is “down” for the closed state and “up” for the pre-open and open states (Tian et al., 2007; Kang et al., 2008; Silva et al., 2009; Strutz-Seebohm et al., 2011). The interaction of KCNE1^{T58} with Kv7.1^{S338} and Kv7.1^{F339} may uncouple the gate at the lower S6 from the S4–S5 linker, which leads to S6 bundle crossing and closure of the gate (Strutz-Seebohm et al., 2011). The formation of the S6 bundle upon S4–S5 linker uncoupling may be supported by the lack of a central S6 gating hinge (Seebohm et al., 2006). The uncoupling of S4–S5 by KCNE1 during closed state inactivation shows similarities to an uncoupling event of the PD from the voltage sensor domain during closed state inactivation of some Kv channels as described by the group of Bähring et al. (2012). In the light of the ongoing discussion about fixed stoichiometry of two KCNE1 subunits per Kv7.1 tetramer vs. variable stoichiometry it is fair to study a channel complex with two KCNE1 subunits docked to four Kv7.1 subunits (Strutz-Seebohm et al., 2011). This stoichiometry is consistent with both hypotheses. Morphing experiments have been performed (Figure 5). As expected the gating is associated with large structural rearrangements in the voltage sensor domain. On the other hand the PD is rather rigid in the region around the selectivity filter. The KCNE1 transmembrane segment undergoes relatively small rearrangements during the gating steps $C \leftrightarrow C^*$ and $O \leftrightarrow C$ but dramatic structural alterations during the $C^* \leftrightarrow O$ transition (Figure 5; the extent of structural rearrangement around key residue KCNE1 T⁵⁸ is indicated by a yellow arrow). This dramatic rearrangement of KCNE1 associated with the $C^* \leftrightarrow O$ transition may generate an energetic barrier that can only slowly be overcome, resulting in dramatically slowed activation, the pivotal characteristic of Kv7.1/KCNE1 gating. Clearly, these models present a preliminary view on the dynamics of Kv7.1/KCNE1 channels. Silva et al. (2009) modeled further structural closed states of the Kv7.1 voltage sensor module. Incorporation of these data in future Kv7.1/KCNE1 morphing experiments may prove valuable. Increase in knowledge combined with the already available data, upcoming improvements in computational methodology, and decreasing computational costs will allow for much more facilitated dynamic modeling of Kv7.1/KCNE1 channel gating in the future.

The novel structural “snapshots” allow for a better understanding of Kv7.1/KCNE1 molecular pharmacology. Several



Kv7.1/KCNE1 inhibitors and activators bind to distinct sites in state-dependent manner (Lerche et al., 2000, 2007; Seebohm et al., 2001b, 2003a; Chen et al., 2003b). Docking of various compounds to the different models and experimental verifications are now possible. An interesting observation is that the KCNE1 transmembrane segment induces large fenestrations in the Kv7.1 channel pre-open state that may allow for a novel access pathway not present in Kv7.1 homomeric channels (Strutz-Seebohm et al., 2011). Affected by these structural effects is the central cavity, the binding and blocking site of several Kv7.1/KCNE1 inhibitors (Lerche et al., 2000, 2007; Seebohm et al., 2003a). This altered structure could be used as a scaffold for the computer-aided design of specific Kv7.1/KCNE1 modulators not targeting homomeric Kv7.1 channels, which would be a prerequisite for tissue specific pharmacology (Seebohm et al., 2003b; Strutz-Seebohm et al., 2011). Insights into KCNE1-specific molecular pharmacology will be highly valuable for both drug development and safety pharmacology alike (Towart et al., 2009; Pugsley et al., 2011).

Upcoming physiological, pathophysiological, pharmacological, and structural analyses will fill the gaps in our current knowledge, and therefore the old Kv7.1/KCNE1 channel faces an exciting future. Let's dance the KCNQ1 tango!

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APPENDIX

Table A1 | Kv7.1 expression in mammalian tissues.

Specified tissue	Species	mRNA/protein	Reference
Brain	Mouse	mRNA	Lee et al. (2000)
	Mouse	mRNA	Ohya et al. (2003)
	Mouse	mRNA	Yeung et al. (2007)
	Mouse	mRNA	Iannotti et al. (2010)
Inner ear	Mouse	mRNA	Neyroud et al. (1997)
	Mouse	mRNA/protein	Nicolas et al. (2001)
	Mouse	Protein	Knipper et al. (2006)
	Rat	mRNA	Liang et al. (2006)
	Rat	Protein	Hur et al. (2007)
	Guinea pig	mRNA	Liang et al. (2006)
Trachea	Mouse	mRNA/protein	Grahammer et al. (2001b)
Thyroid gland	Mouse	mRNA/protein	Frohlich et al. (2011)
	Human	mRNA	Yang et al. (1997)
	Human	mRNA	Frohlich et al. (2011)
Thymus	Mouse	mRNA	Demolombe et al. (2001)
	Human	mRNA	Chouabe et al. (1997)
Lung	Mouse	mRNA	Demolombe et al. (2001)
	Human	mRNA	Wang et al. (1996b)
	Human	mRNA	Sanguinetti et al. (1996)
	Human	mRNA	Chouabe et al. (1997)
	Human	mRNA	Yang et al. (1997)
Heart	Mouse	mRNA	Barhanin et al. (1996)
	Mouse	mRNA	Lee et al. (2000)
	Mouse	mRNA	Casimiro et al. (2001)
	Mouse	mRNA	Demolombe et al. (2001)
	Mouse	mRNA	Ohya et al. (2003)
	Mouse	mRNA	Yeung et al. (2007)
	Mouse	mRNA	Iannotti et al. (2010)
	Mouse	mRNA	Strutz-Seeböhm et al. (2006)
	Mouse	Protein	Knollmann et al. (2007)
	Rat	Protein	Rasmussen et al. (2004)
	Guinea pig	mRNA/protein	Zicha et al. (2003)
	Guinea pig	Protein	Nicolas et al. (2008)
	Ferret	mRNA	Brahmajothi et al. (1997)
	Rabbit	mRNA/protein	Zicha et al. (2003)
	Horse	Protein	Finley et al. (2002)
	Canine	mRNA/protein	Han et al. (2002)
	Human	mRNA	Wang et al. (1996b)
	Human	mRNA	Sanguinetti et al. (1996)
	Human	mRNA	Chouabe et al. (1997)
	Human	mRNA	Yang et al. (1997)
Human	mRNA	Bendahhou et al. (2005)	
Human	mRNA	Gaborit et al. (2007)	
Human	mRNA	Frohlich et al. (2011)	
Human	mRNA/protein	Zicha et al. (2003)	
Liver	Mouse	mRNA	Lee et al. (2000)
	Mouse	mRNA	Demolombe et al. (2001)
Spleen	Mouse	mRNA	Demolombe et al. (2001)
	Human	mRNA	Chouabe et al. (1997)

(Continued)

Table A1 | Continued

Specified tissue	Species	mRNA/protein	Reference
Pancreas	Mouse	mRNA	Demolombe et al. (2001)
	Rat	Protein	Lee et al. (2008)
	Human	mRNA	Sanguinetti et al. (1996)
	Human	mRNA	Chouabe et al. (1997)
	Human	mRNA	Yang et al. (1997)
Stomach	Mouse	mRNA	Lee et al. (2000)
	Mouse	mRNA	Demolombe et al. (2001)
	Mouse	mRNA/protein	Dedek and Waldegger (2001)
	Mouse	Protein	Grahammer et al. (2001a)
	Mouse	Protein	Heitzmann et al. (2004)
	Mouse	Protein	Heitzmann and Warth (2007)
	Rat	mRNA/protein	Lambrecht et al. (2005)
	Human	mRNA	Yang et al. (1997)
	Human	mRNA	Frohlich et al. (2011)
	Human	Protein	Grahammer et al. (2001a)
	Colon	Mouse	mRNA/protein
Rat		mRNA	Kunzelmann et al. (2001)
Human		mRNA	Chouabe et al. (1997)
Human		mRNA	Yang et al. (1997)
Human		mRNA	Frohlich et al. (2011)
Small intestine	Human	mRNA/protein	Horikawa et al. (2005)
	Mouse	mRNA	Demolombe et al. (2001)
	Mouse	mRNA/protein	Dedek and Waldegger (2001)
	Human	mRNA	Chouabe et al. (1997)
Kidney	Human	mRNA	Yang et al. (1997)
	Mouse	mRNA	Barhanin et al. (1996)
	Mouse	mRNA	Lee et al. (2000)
	Mouse	mRNA	Demolombe et al. (2001)
	Mouse	Protein	Vallon et al. (2001)
	Human	mRNA	Wang et al. (1996b)
	Human	mRNA	Sanguinetti et al. (1996)
	Human	mRNA	Chouabe et al. (1997)
	Human	mRNA	Yang et al. (1997)
	Human	mRNA	Yang et al. (1997)
Uterus	Mouse	mRNA/protein	McCallum et al. (2009)
Placenta	Human	mRNA	Wang et al. (1996b)
	Human	mRNA	Sanguinetti et al. (1996)
	Human	mRNA	Chouabe et al. (1997)
	Human	mRNA	Yang et al. (1997)
Ovary	Human	mRNA	Chouabe et al. (1997)
Prostate	Human	mRNA	Chouabe et al. (1997)
	Human	mRNA	Yang et al. (1997)
Testis	Human	mRNA	Chouabe et al. (1997)
Blood vessels	Mouse	mRNA/protein	Ohya et al. (2003)
	Mouse	mRNA/protein	Yeung et al. (2007)
	Rat	mRNA	Brueggemann et al. (2007)
	Rat	mRNA	Mackie et al. (2008)
	Rat	mRNA	Joshi et al. (2009)
	Rat	mRNA/protein	Zhong et al. (2010)
	Human	mRNA/protein	Ng et al. (2011)
	Human	mRNA	Demolombe et al. (2001)
Skeletal muscle	Mouse	mRNA	Demolombe et al. (2001)
	Mouse	mRNA	Iannotti et al. (2010)
	Rat	mRNA	Roura-Ferrer et al. (2008)
Peripheral blood leukocytes	Human	mRNA	Chouabe et al. (1997)
Hematopoietic stem cells	Human	mRNA	Park et al. (2011)