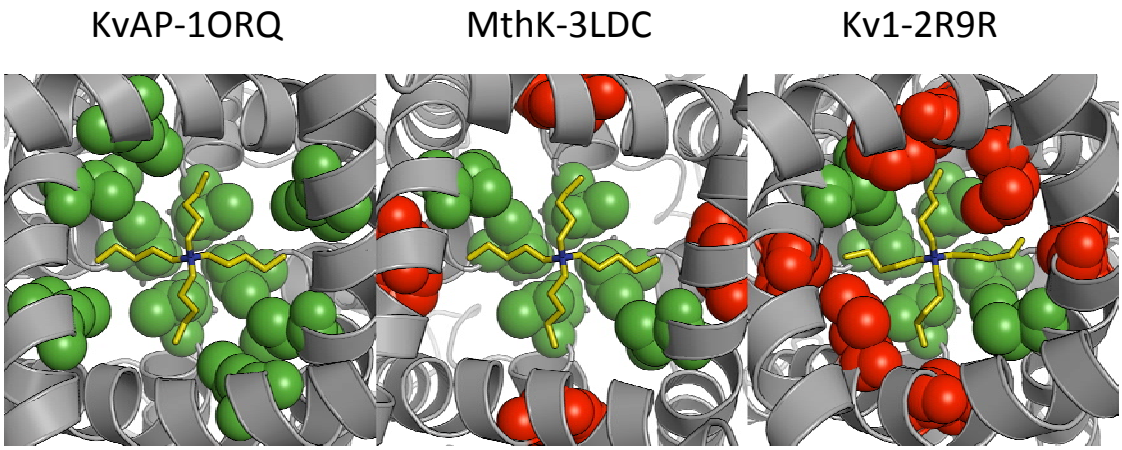
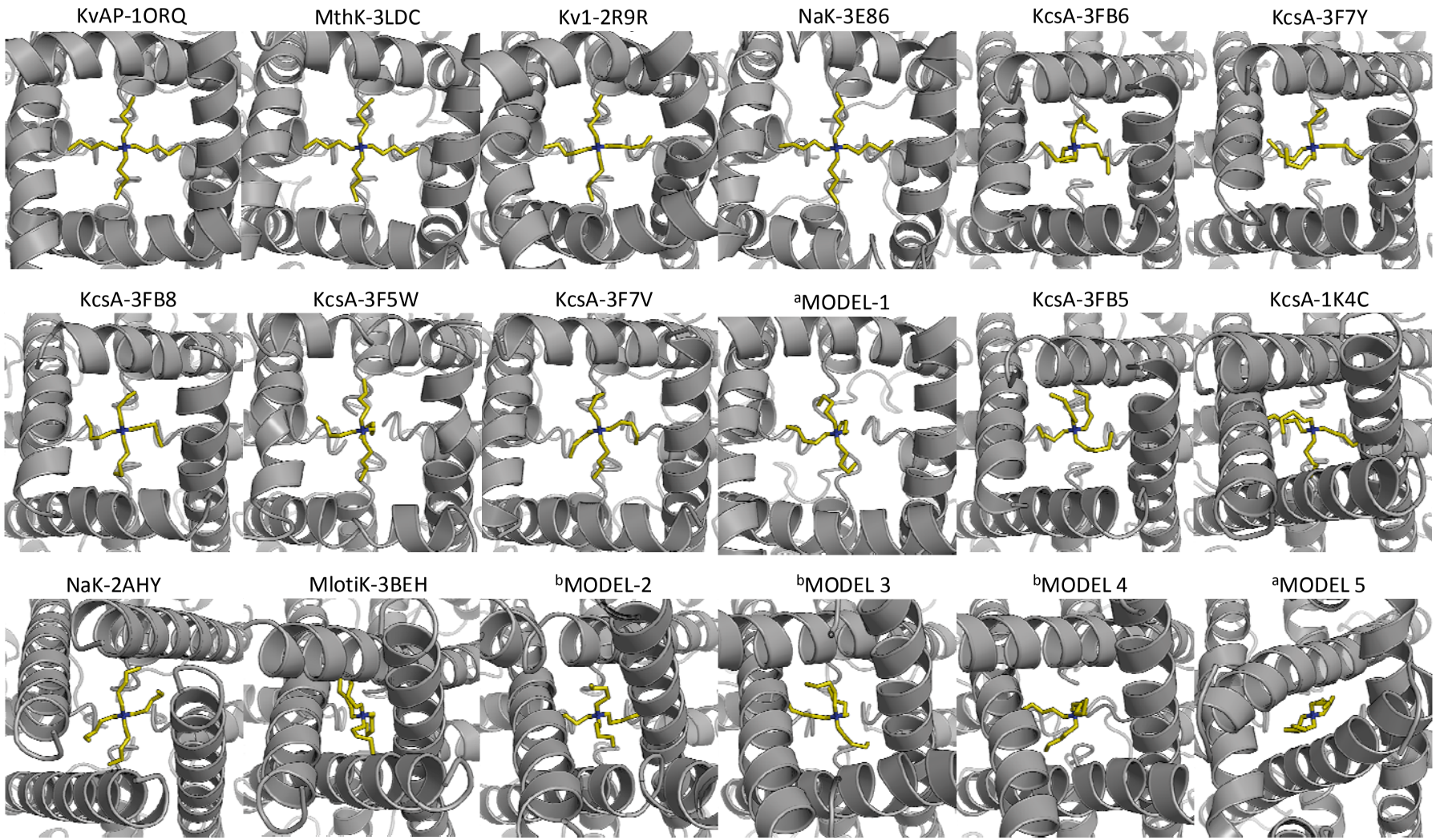
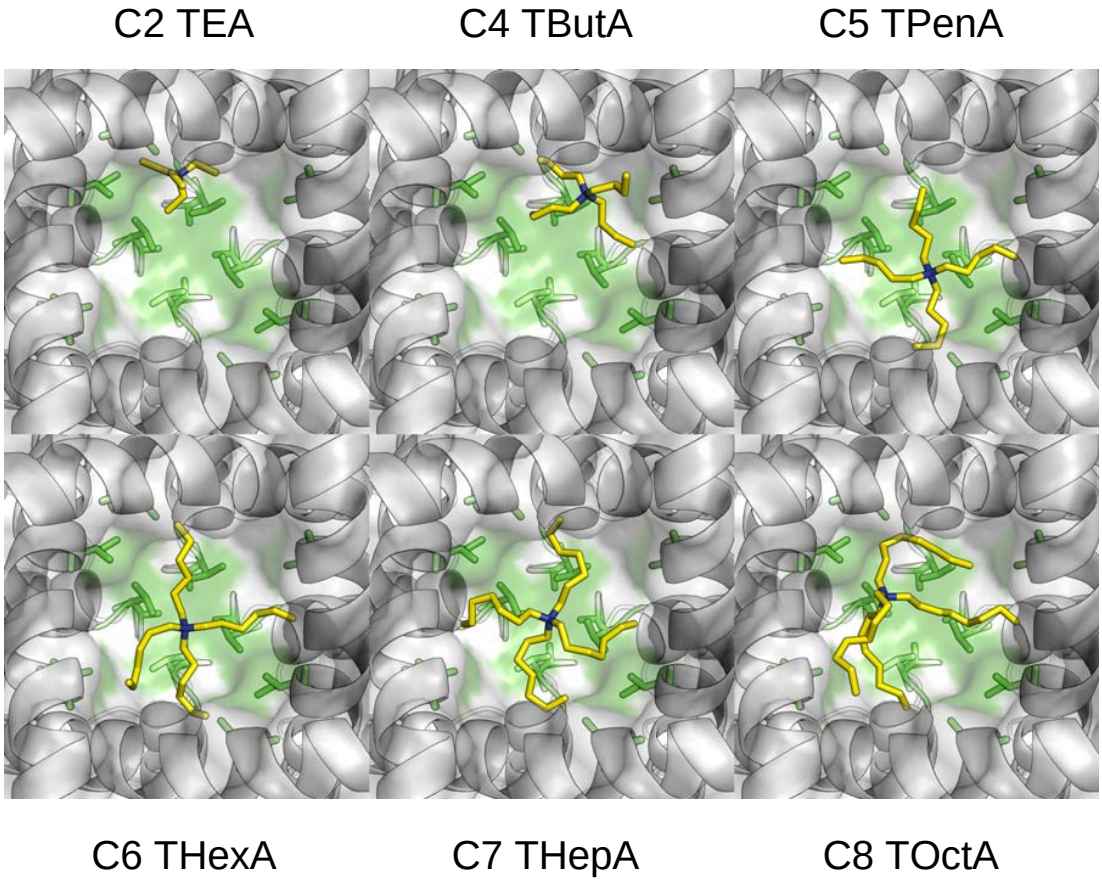


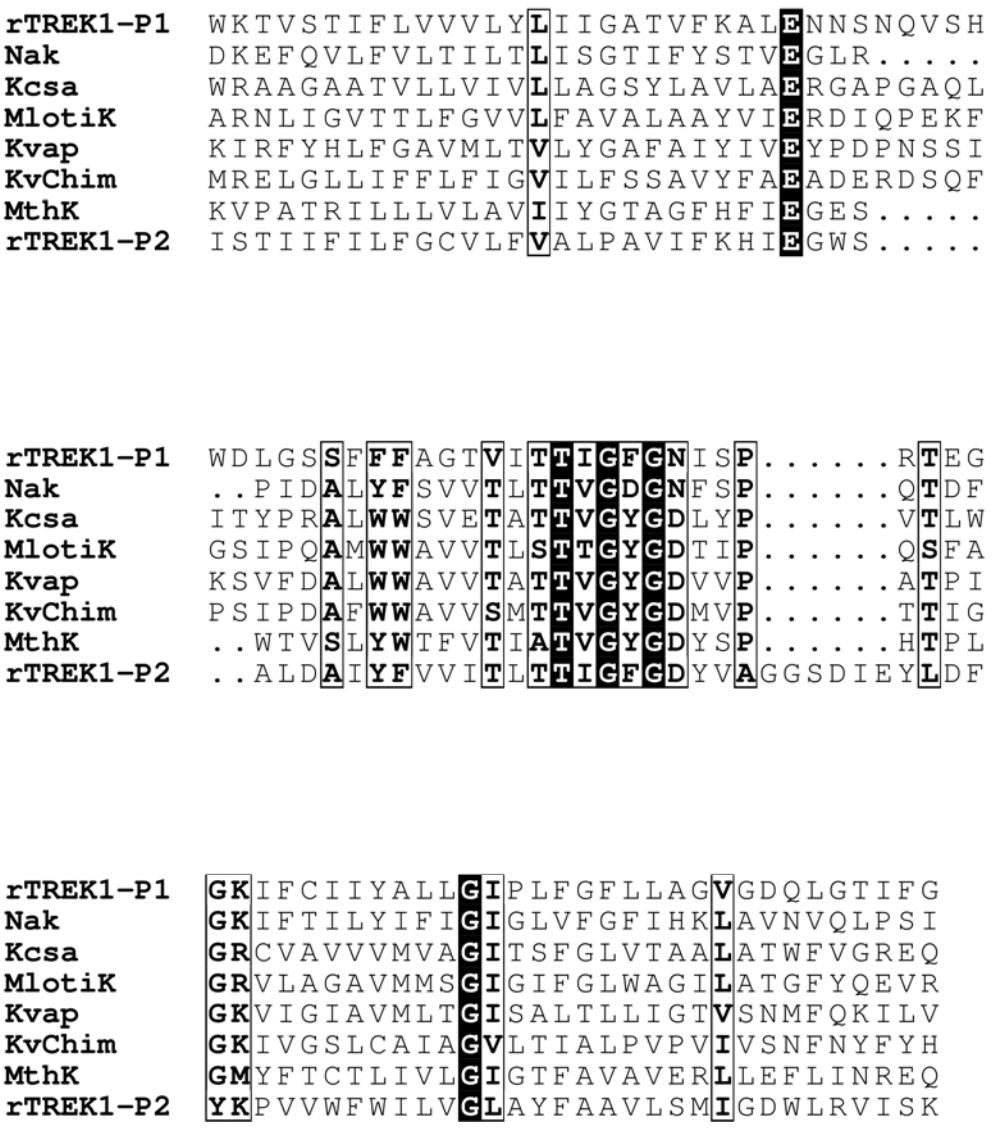
Supplementary Figure S1: Detailed view of TPenA docked into the 3 best-fit homology models of TREK-1. In green are shown residues in the TREK-1 model which come within 4Å of the docked TPenA and which correlate with data obtained from the functional mutagenesis screen (Figure 3 and Table1). In red are shown residues which are predicted to interact when TPenA is docked into the model, but which show no functional effect on TPenA block when mutated. In all three models (which are based upon open state structures) TPenA adopts an extended configuration when docked into the pore, similar to that seen for TButA in KcsA (PDB 1J95). However, the model based upon the KvAP structural template has the highest number of hits and no false-positives. The next best models have increasing numbers of false-positives predicted by their proximity to the TPenA molecule.



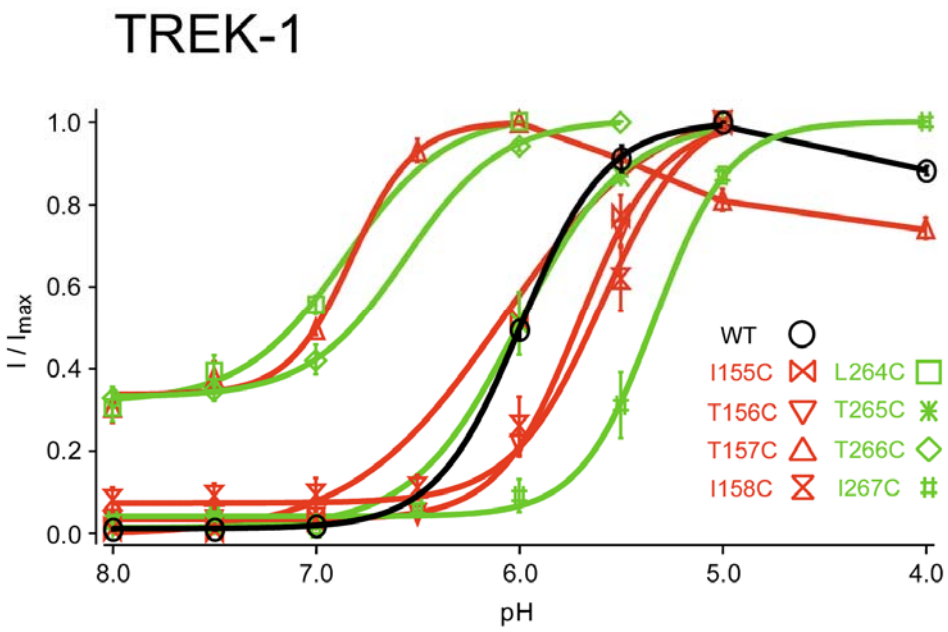
Supplementary Figure S2: TPenA docked into 18 different homology models of the TREK-1 channel pore based upon the structural templates indicated (pdb codes shown). The models were ranked from top-left according to the scheme shown in Table 1. The best-fit models are primarily based upon open-state structures, whereas models based upon other structural templates result in TPenA being unable to adopt a configuration consistent with the functional mutagenesis. Models 1-5 are structural models of TREK-1 that have been published previously; [a]Milac *et al* (2011) [b]Treptow & Klein (2010).



Supplementary Figure S3: Flexible ligand dockings of different size QA ions into the best-fit (KvAP) TREK-1 homology model, using Autodock4. Residues predicted to form part of the TPenA binding site are shown in green. Only when the alkyl tail of the QA ion reaches at least five carbon atoms in length (C5 TPenA) is the nitrogen atom able to bind centrally within the cavity, whilst still allowing the tails make contacts with all four cavity walls. However, as the chain length increases beyond C6 THexA the QA ion does not fit in an extended planar configuration e.g. C8 TOctA appears too large to be optimally accommodated within the central cavity without internal steric clashes. These *in silico* results agree well with the chain length dependence of K2P channel block shown in Figure 1.



Supplementary Figure S4: The alignment of TREK-1 with other channels used to produce the homology models shown in Table 1.



Supplementary Figure S5: Sensitivity of mutations near the selectivity filter affect pHi gating in TREK-1. pH-dose response curves of the proton activated current for TREK-1 WT and mutant channels located in P1 (red) or P2 (green) fitted with a standard Hill equation. Fits were constrained between the current at pH 8.0 and the maximal current level that varied according to the shift in pH sensitivity (i. e. at pH 5.0 for TREK-1 WT and at pH 6 for TREK-1 T157C). The proton dependent inhibition was observed for all channels, but for clarity is only shown for WT TREK-1 (black) and TREK-1 T157C (red) where proton inhibition initiates earlier according to the shift in pH-EC_{50}.

		in vitro	KvAP	MthK	Kv1	NaK	KcsA	KcsA	KcsA	KcsA	KcsA	Model1	KcsA	KcsA	NaK	MlotiK	Model-2	Model-3	Model-4	Model-5
			1ORQ	3LDC	2R9R	3E86	3FB6	3F7Y	3FB8	3F5W	3F7V	a	3FB5	1K4C	2AHY	3BEH	b	b	b	a
			Open	Open	Open	Open	Open 16	Open 17	Open 20	Open 32	Open 23	Open	Open 14	Closed	Closed	Closed	Closed	Closed	Closed	Closed	Closed
P1	T156																	✗	✗	
	T157	●	✓	✓	✓	✓	✓		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	
	I158																			
TM2	I182	●	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓			✓	✓		
	P183	●																		
	L184																		✗	✗
	F185			✗	✗			✗	✗	✗		✗		✗	✗	✗	✗	✗	✗	✗
	G186																	✗		
	F187																			
	L188																			
	L189	●	✓													✓		✓	✓	✓
	A190																			✗
P2	T265																✗	✗	✗	
	T266	●	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓					✓
	I267																			
TM4	I293																✗	✗	✗	✗
	L294																		✗	
	V295																			
	G296	●																		
	L297				✗	✗	✗	✗	✗	✗	✗	✗	✗	✗		✗		✗	✗	✗
	A298	●																		
	Y299	●																		
	F300			✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗				✗
	A301					✗	✗					✗	✗							
	A302																			
	V303																			
	L304	●	✓					✓						✓						
	S305	●																		
	M306																			
	I307	●																		
	G308																			
	D309	●																		
	W310																			

Piechotta et al Supplementary Information Table S1

Scoring of Different Homology Models

TPenA was docked into 18 different homology models either created as part of this study or recently published models ([a]Milac et al 2011, [b]Treptow & Klein, 2010). Models were scored according to whether any part of the indicated side chains are ≤ 4Å distance of any part of the docked TPenA molecule. Green tick indicates agrrement with the functional scanning mutagenesis, whereas red cross indicates an interaction predicted in silico but where no functional effect is seen upon mutagenesis. The 'in vitro' column reflects the functional mutagenesis shown in Figure 3A and those highlighted in yellow are not predicted to directly form part of the TPenA binding site (See Figure 4).

Supplementary files also include:

Supplementary Movie: Rotating image of TPenA (yellow) docked into the best-fit homology model of TREK-1. Residues thought to contribute directly to the TPenA binding site are shown in green.

Supplementary Coordinates: PDB file of the best fit model of TREK-1 based upon KvAP (1ORQ) as a structural template. Includes TPenA in optimally docked configuration.