TWIK-related acid-sensitive K^+ channel 1 (TASK-1)

A Target Enabling Package (TEP)

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Therapeutic Area(s)	Neurological diseases
Disease Relevance	Loss of function mutations in TASK-1 read to primary pulmonary hypertension type 4 (PPH4), which is often fatal in mid-life (1). TASK-1 is a K2P channel that helps regulate the resting membrane potential in neurons. It is targeted both for atrial fibrillation and obstructive sleep apnea.
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SUMMARY OF PROJECT

The TWIK related acid-sensitive K^+ channel 1 (TASK-1) belongs to the family of two-pore domain potassium (K_{2P}) channels. It regulates resting membrane potential and is expressed in cardiomyocytes, neurons and vascular smooth muscle cells. Loss of function mutations in TASK-1 lead to primary pulmonary hypertension type 4 (PPH4) which is often fatal in mid-life (1). We have produced TASK-1 and determined structures of this protein alone and in complex with two highly potent inhibitors, BAY 1000493 and BAY 2341237, with EC_{50} values of 9.5 nM and 7.6 nM, respectively. We have used a two-electrode voltage clamp assay to

measure the effect of mutations in TASK-1 and the effect of inhibitors. The native structure of TASK-1 also allowed us to map the six known disease mutations leading to PPH4.

SCIENTIFIC BACKGROUND

TASK-1, the product of the KCNK3 gene, is a two-pore domain potassium channel (K_{2P}), expressed in the lung, placenta and kidneys, where it regulates resting membrane potential and contributes to arterial relaxation (2-4). In contrast to many other potassium channels, TASK-1 is a leak channel, but is highly sensitive to changes in pH within the physiological range (2). In addition, TASK-1 is regulated by a number of other physiological and pharmacological stimuli, for example G proteins, lipids, activators such as volatile anaesthetics and inhibitors A1899 and ML365 (5-8). The mode of action of many of these stimuli remains poorly understood. TASK-1 assembles as a homodimer, with a pseudo-tetrameric transmembrane domain and an extracellular cap domain that is common to members of the K_{2P} family. In addition, it can form heterodimers with other K_{2P}s, most notably its closest homologue, TASK-3 (9).

Primary pulmonary arterial hypertension type 4 (PPH4)

Exome sequencing has identified six mutations in TASK-1 in patients suffering from PPH4 (1). All mutants were found to cause significantly reduced channel currents. The disease is autosomal dominant, with reduced penetrance. It is characterised by increased pulmonary arterial pressure in the absence of common causes of hypertension such as chronic heart or lung disease. Although treatments have improved, affected carriers may require a lung transplant and the disease is often fatal in mid-life.

Birk-Barel syndrome

Mutations in TASK-3, the closest homologue of TASK-1, was found to abolish potassium currents (10). Symptoms of Birk-Barel syndrome include mental retardation, hypotonia and dysmorphism. Due to paternal splicing of the gene encoding TASK-3, the disease is maternally imprinted.

RESULTS – THE TEP

Purification

Human TASK-1, residues M1-E259, with C-terminal deca-histidine and FLAG tags, preceded by a tobacco etch virus (TEV) protease cleavage site, was expressed using the baculovirus/Sf9 cell system. The protein was extracted using the detergent n-decyl-β-D-maltopyranoside (DM) supplemented with cholesteryl hemisuccinate (CHS) in a 10:1 wt:wt ratio and subsequently purified in 0.24% w/v DM and 0.024% CHS using TALON resin, TEV cleavage, reverse purification and size exclusion chromatography. The final size exclusion buffer contained 20 mM HEPES pH 7.5, 200 mM KCl, 0.12% w/v DM and 0.012% w/v CHS. After purification, the protein was concentrated to 10-30 mg/ml and the final yield per litre of initial culture volume was 0.4 mg.

Crystal structures

TASK-1 X-ray data and structures obtained:

- 3.0 Å native structure (PDB: 6RV2)
- 2.9 Å BAY 1000493 complex (PDB: 6RV3)
- 3.1 Å BAY 2341237 complex (PDB: 6RV4)

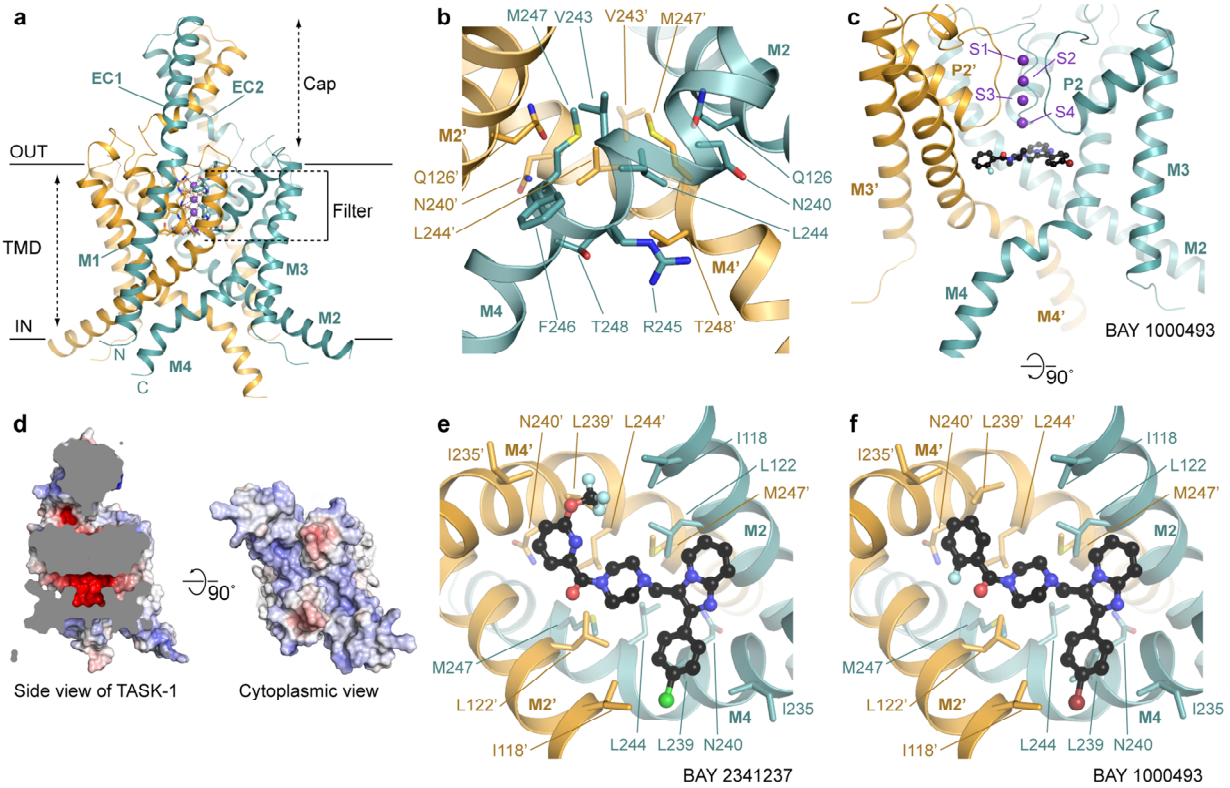

Fig. 1 (a) The TASK-1 Apo Structure, with a closed gate shown in **(b)**. **(c)** The BAY 1000493 complex, showing the compound bound in the vestibule. **(d)** Surface representation of the apo structure shown from the membrane plane and rotated 90°. View of the compound binding site shown from the selectivity filter looking towards the cytoplasmic gate with **(e)** BAY 2341237 and **(f)** BAY 1000493 shown as ball and stick models.

We determined the structure of TASK-1 to 3.0 Å resolution (PDB: 6RV2) (Figure 1a). The structure shows that TASK-1 forms a domain-swapped heterodimer, with four transmembrane helices per monomer (M1 to M4). The transmembrane region forms a pseudotetrameric selectivity filter comprised of pore loops P1 and P2 from each subunit, and closely resembles previous K_{2P} structures of TWIK-1 (11), TRAAK (12), TREK-2 (13) and TREK-1 (PDB: 4TWK and (14)). Furthermore, the structure is in a down-state conformation and the central vestibule is open to the membrane via lateral fenestrations lined with hydrophobic residues. Notably, K_{2P} channels have a large extracellular domain consisting of helices EC1 and EC2 from each subunit. In all available K_{2P} structures, the cap is held together by a disulphide bridge at the cap apex, but this is missing in TASK-1, TASK-3 and TASK-5. We show that the domain swap and cap fold remain intact despite the lack of a disulphide bridge.

Most notably, the M4 helices of TASK-1 bend at L241, to form an antiparallel gate towards the cytoplasm, using residues V243-T248. This region was previously known as the halothane response element (sequence VLRFMT). This unusual conformation causes the pore to be blocked from the cytoplasm and the structure is clearly in a closed conformation. After the gate, the M4 helices from each monomer bend again and form a network of polar and charged interactions with their own M1 and M2 from the other monomer. Mutations in this region result in a markedly increased channel activity, confirming that this region is important for opening and closing of the gate.

The TASK-1 structures in complex with BAY 1000493 and BAY 2341237, determined to 2.9 Å and 3.1 Å respectively (PDB ID: 6RV3 and 6RV4), show that both small molecule inhibitors bind directly below the selectivity filter and are trapped in the vestibule by the closed cytoplasmic gate. This may explain the high potency of these inhibitors.

Disease associated mutations

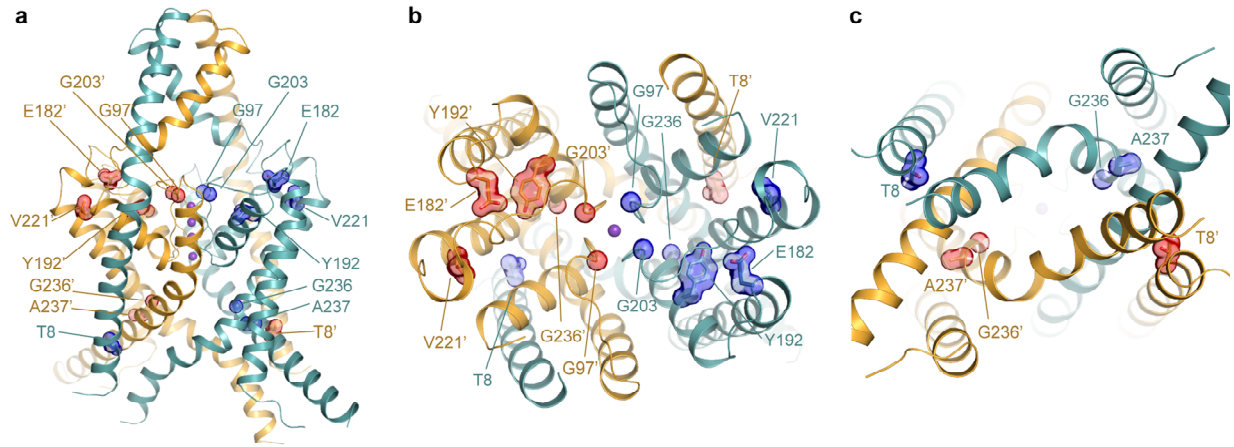

Fig. 2 Mutations found in PPH4 and Birk-Barel syndrome (blue/red) mapped onto the TASK-1 structure, shown from **(a)** the membrane plane, **(b)** looking down from the cap towards the selectivity filter and **(c)** from the cytoplasm.

TASK-1 PHH4 disease associated mutations

To date, six mutations in TASK-1 have been identified in patients suffering from primary pulmonary hypertension (1) (Figure 2). They are all thought to result in loss of channel function. Out of these mutations, two are located at the extracellular side just after the selectivity filter (G97R and G203D). These mutations likely influence the pore conformation and may result in blocking of the channel. Y192C and V221L are buried in the hydrophobic core of the protein and in the case of V221L are close to the lipid bilayer and thus, mutation of this residue likely destabilises the protein. E182K at the end of M3 interacts with the loop following the selectivity filter and may affect conformation of this region. Interestingly, the T8K mutation is located in the region of M1 that interacts with M4 through a network of hydrogen bonds and this mutation potentially increases the strength of these interactions, leading to an increased probability of a closed channel.

TASK-3 and Birk-Barel syndrome

As mentioned previously, the maternally imprinted mental disorder Birk-Barel syndrome is a result in a loss of function mutation in TASK-3, G236R (10). Due to the close similarity between TASK-1 and TASK-3, we could map the location of this residue on our structure (Figure 2). The G to R mutation would lead to a large positively charged residue pointing towards the centre of the vestibule, possibly obstructing the passage of potassium ions through the vestibule. Recently an adjacent mutation A237D, has been identified in a patient with Birk-Barel syndrome (15). This change places a larger charged residue in a confined hydrophobic space, an arrangement that is likely to destabilise TASK-1 or prevent proper folding.

Chemical matter

- Two high affinity TASK-1 inhibitors have been developed at Bayer in Germany:
  - BAY 1000493: EC_{50} = 9.5 nM
  - BAY 2341237: EC_{50} = 7.6 nM

Biophysical assays

Through collaboration with Prof. Niels Decher (University of Marburg, Germany) we have implemented a two-electrode voltage clamp assay to measure the activity of TASK-1.

- **Two-electrode voltage clamp assay**

Our collaborators developed a TEVC assay to study the effect of mutations in TASK-1 as well as compound binding. They were able to confirm which residues in TASK-1 interact with the two compounds. The assay measures current through TASK-1 expressed in *Xenopus laevis* oocytes.

Using this assay, we performed scanning mutagenesis across the proximal M1, distal M3 and distal M4 helices to investigate the role of residues comprising the gate as well as the surrounding residues. Many of these mutations resulted in strong gain of function phenotypes, which is consistent with opening of the cytoplasmic gate.

We also demonstrated that both BAY 1000493 and BAY 2341237 inhibit TASK-1 and that they interact with residues in the vestibule, notably L122 and L239.

In addition, we showed that mutations in the gate, for example L244A, did not impact compound affinity but led to a faster wash out of the compound indicating that the cytoplasmic gate must open in order for the compound to dissociate from TASK-1.

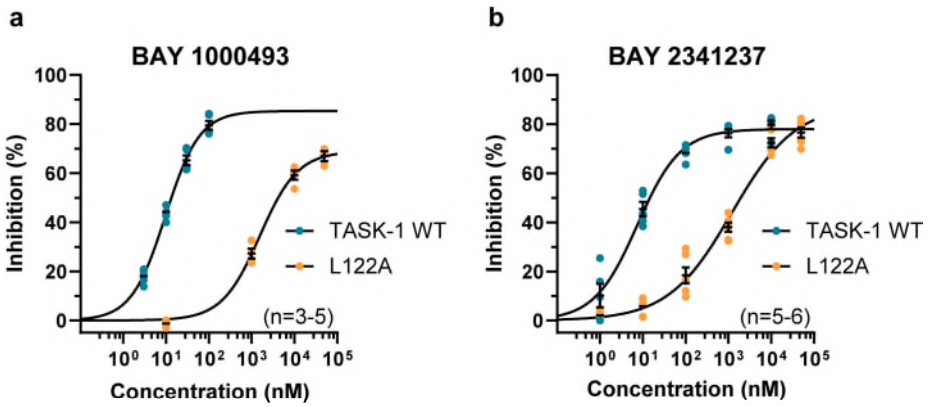

Fig. 3 Dose-response curves of TASK-1 inhibitors (a) BAY 1000493 and (b) BAY 2341237, data from Niels Decher's lab.

CONCLUSION

Biological understanding

- TASK-1 forms a domain-swapped homodimer despite lack of a disulphide bond at the cap apex
- The structure of TASK-1 in a **closed** conformation reveals a unique **lower gate** found so far only in TASK channels
- We show how **BAY 1000493 and BAY 2341237** bind and inhibit TASK-1
- The presence of the lower gate **explains the very slow washout rates** seen for inhibitors of TASK channels.
- There are **six mutations found in PPH4 patients**. Mapping the mutations on the structure has allowed us to identify the likely mechanisms for loss of activity

Medical relevance

- Structures may help understanding how inhibitors act on TASK-1.
- Design of compounds that can distinguish between TASK-1 and TASK-3, which could help understanding the physiological roles of TASK homo- and heterodimers.
- TASK-1 specific compounds are already in clinical trials for sleep apnea and atrial fibrillation.
- There are currently no known high affinity TASK-1 activators, although all known PPH4 causing mutations results in a loss of function. Understanding the mechanisms of TASK-1 activation may help in design of specific TASK-1 activators.

Collaborations

The following collaborations have been established:

- **Niels Decher (University of Marburg, Marburg, Germany)** – Studies function of TASK channels
- **Thomas Mueller (Bayer Pharma AG, Wuppertal, Germany)** – Develops TASK-1 inhibitors
- **Stephen Tucker (Dept. of Physics, Oxford)** – K2P channel function

TEP IMPACT

- Manuscript in BioRxiv: https://doi.org/10.1101/706168
- The compounds described in this work are included in patent no WO2017097792A1 held by Bayer.
- We will continue to collaborate with Thomas Mueller, (Bayer Pharma AG), Niels Decher (Marburg) and Stephen Tucker (Oxford) to further our understanding of how the X-gate controls the function of TASK-1.
- TASK-1 inhibitors are currently in clinical trials for obstructive sleep apnea and atrial fibrillation.

FUNDING INFORMATION

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ADDITIONAL INFORMATION

Structure Files

PDB ID	Structure Details	Resolution (Å)
6RV2	Native TASK-1	3.0
6RV3	Complex with TASK-1 and inhibitor BAY 1000493	2.9
6RV4	Complex with TASK-1 and inhibitor BAY 2341237	3.1

Materials and Methods

Protein Expression and Purification

Vector: pFB-CT10HF-LIC (available from The Addgene Nonprofit Plasmid Repository)
Cell line: DH10Bac, Sf9 cells
Tags and additions: C-terminal TEV protease site, followed by 10x His and FLAG tags

Wild-type sequence:
MKRQNVRTLALIVCTFTYLLVGAAVFDALESEPELIERQRLELRQQELRARYNLSQGGYEELERVVLRLKPHKAGVQWRFA
GSFYFAITVITTIGYGHAAPSTDGGKVFCMFYALLGIPLTLVMFQSLGERINTLVRYLLHRAKKGLGMRRADVSMANMVLI
GFFSCISTLCIGAAAFSHYEHWTFFQAYYYCFITLTTIGFGDYVALQKDQALQTQPQYVAFSFVYILTGLTVIGAFLNLVVLRF
MTMNAEDEKRDAENLYFQSHHHHHHHHHHDYKDDDDK
(underlined sequence contains vector encoded TEV protease cleavage site, His and FLAG tag)

Expression
The human KCNK3 gene (Genbank ID 4504849), which encodes the TASK-1 protein, was obtained from Origene. The crystallisation construct, comprising residues M1-E259 was subcloned into the pFB-CT10HF-LIC vector and baculovirus was generated using the Bac-to-Bac system. Briefly, this was performed by transforming into *Escherichia coli* strain DH10Bac to generate bacmid DNA, which was subsequently used to transfect *Spodoptera frugiperda* (Sf9) insect cells and generate recombinant baculovirus. Large scale grow-ups of Sf9 cells were infected with baculovirus and incubated for 72 h at 27 °C in shaker flasks.

Cell Lysis and detergent extraction of membrane proteins
Extraction Buffer (EXB): 50 mM HEPES pH 7.5, 200 mM KCl, 5% v/v glycerol, Roche protease inhibitors

The cell pellet from 1 litre of insect cell culture was resuspended in 40 ml of lysis buffer per litre pellet using a glass dounce homogeniser. Cells were lysed with an EmulsiFlex-C3 or C5 homogenizer (Avestin Inc.) at 15,000 psi, two passes. For solubilisation, 5 ml of 10%/1% w/v stock of DM/CHS was added per litre of cell culture and the volume was adjusted by the addition of EXB to a final volume of 50 ml/L of initial cell culture and rotated at 4°C for 1 hour. The insoluble fraction was removed by centrifugation at 35,000 g for 1 hour at 4°C.

Purification
Wash Buffer: 50 mM HEPES pH 7.5, 10 mM imidazole pH 8.0, 200 mM KCl, 5% w/v glycerol, 0.24% w/v DM, 0.024% w/v CHS
Elution Buffer: 50 mM HEPES pH 7.5, 250 mM imidazole pH 8.0, 200 mM KCl, 5% v/v glycerol, 0.24% w/v DM, 0.024% w/v CHS
PD10 Buffer: 50 mM HEPES pH 7.5, 200 mM KCl , 5% w/v glycerol, 0.24% w/v DM, 0.024% w/v CHS
Size exclusion buffer (SEC) Buffer: 20 mM HEPES pH 7.5, 200 mM KCl, 0.12% w/v DM, 0.012 % CHS

Column 1: Co^{2+} TALON resin (0.5 ml volume in a gravity-flow column)
The detergent-extracted membrane protein from each litre of cells was combined with 1 ml of pre-equilibrated slurry of 50% Co^{2+} TALON resin (previously washed twice with H_2O and three times with EXB). Imidazole pH 8.0 was added to a final concentration of 5 mM and the sample was rotated for 1 hour at 4°C.

It was then transferred to a gravity column and washed with 30 column volumes of wash buffer. The sample was eluted with two column volumes of elution buffer in 0.5-2 ml fractions. Peak fractions were combined and passed through PD10 columns, pre-equilibrated in PD10 buffer (one column per 5 litres of initial culture volume).

TEV protease cleavage and reverse purification

TEV protease and PNGaseF were added at ratios of 5:1 and 10:1 (TASK-1:enzyme, wt:wt), respectively, and incubated at 4°C overight. For each litre of initial cell culture volume, 0.2 ml of a 50 % slurry of TALON resin (pre-equilibrated as above) were added and the sample was rotated in the cold room for 1 hour. The sample was transferred to a gravity column and the flow-through was collected.

Column 2: Superose 6 Increase 10/300 GL column (GE Healthcare)

The protein sample was concentrated in a 100 kDa MWCO concentrator (pre-equilibrated in SEC buffer without detergent) at 4,000 g with mixing every 5 min, to a final volume of 500 µl. After centrifugation at 20,000 g for 30 min at 4°C, the sample was subjected to size exclusion chromatography on a Superose 6 Increase 10/300 column, previously equilibrated in SEC buffer. The peak fractions were pooled and concentrated in a 100 kDa MWCO concentrator as described previously. After concentration to 10-30 mg/ml, the protein was flash-frozen in liquid nitrogen.

Assays

Two-electrode voltage clamp assay

X. laevis oocytes were obtained and the TEVC measurements were recorded as described previously (7). Briefly, collected oocytes were stored at 18 °C in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES pH 7.5) supplemented with 50 mg/l gentamicin, 274 mg/l sodium pyruvate and 88 mg/l theophylline. Oocytes were injected with 5 ng of TASK-1 cRNA and incubated for 48 h at 18 °C. ND96 was used as the recording solution. Oocytes were held at -80 mV and voltage was ramped from -120 to +45 mV within 3.5 s, using a sweep time interval of 4 s. Block was analysed with voltage steps from a holding potential of -80 mV. A first test pulse to 0 mV of 1 s duration was followed by a repolarising step to -80 mV for 1 s, directly followed by another 1 s test pulse to +40 mV. The sweep time interval was 10 s. All inhibitors were dissolved in DMSO, aliquoted, stored at -20 °C or room temperature and added to the external solution (ND96) just before the recordings. The EC_{50} was determined from Hill plots using four concentrations for each construct. The final DMSO concentration of 0.1 % was not exceed.

Structure Determination

Crystallisation

Crystals were grown using the HiLiDe method (16). Prior to crystallisation, 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) in chloroform was dried down in a round-bottomed glass vial. A total amount of 1.5 µg lipid per µl protein was used. Subsequently, protein at 5.5-6.5 mg/ml was added along with 15 µg/µl DM and 1.5 µg/µl CHS (from a 10% w/v DM, 1% w/v CHS stock). The vial was incubated slowly shaking at 4°C for 16-24 h and centrifuged at 15,000 g for 2 hours at 4°C. Crystallisation trials were then set up in 24-well hanging drop plates with 2 µl drops and a 2:1 protein:reservoir ratio at 20°C. Crystallisation of TASK-1/compound complexes was undertaken in the same manner. Each compound was dissolved in 100% DMSO at 130 mM then added to the HiLiDe setup to a final concentration of 1.3 mM. Crystals grew over 1-12 weeks, in 0.1 M TRIS pH 8.5, 0.05 M KCl, 24-32% PEG400 and 3% w/v sucrose and were mounted at 6°C directly from the drop and vitrified in liquid nitrogen.

Data collection and structure determination

All data were collected at Diamond Light Source (beamline I24) to resolutions between 2.9-3.5 Å. Data were processed, reduced and scaled using XDS, Staraniso and AIMLESS.

The crystals belonged to space group $P22_12_1$ and contained two copies of TASK-1 homodimers in the asymmetric unit. Initial phases were obtained with molecular replacement, using Phaser. A truncated

version of the TREK-2 structure (PDB: 4BW5) (13) was used as an initial search model, with the cap and TM domain separate. An initial TASK-1 model was built using a density modified prime-and-switch map calculated using *phenix.autobuild* as a guide and was improved by several rounds of manual model building and refinement in COOT. The final model was refined in BUSTER version 2.10.3.

The native structure was used for the first round of refinement against the BAY 1000493 and BAY 2341237 data. Only minor changes in the structures were observed. A large positive difference density was observed in the vestibule, under the pore, originating from the compounds. The BAY 1000493 compound was modelled in two alternative conformations, a consequence of the two-fold symmetry of the TASK-1 homodimer. This dual orientation was confirmed by anomalous data collected at the Bromine edge. The BAY 2341237 compound induced small changes in the conformations of vestibule residues, leading to a slight asymmetry between the two monomer chains and BAY 2341237 only binding in one orientation that was clearly resolved in the density.

References

1. Ma, L., Roman-Campos, D., Austin, E. D., Eyries, M., Sampson, K. S., Soubrier, F., Germain, M., Tregouet, D. A., Borczuk, A., Rosenzweig, E. B., Girerd, B., Montani, D., Humbert, M., Loyd, J. E., Kass, R. S., and Chung, W. K. (2013) A novel channelopathy in pulmonary arterial hypertension. *The New England journal of medicine* **369**, 351-361
2. Duprat, F., Lesage, F., Fink, M., Reyes, R., Heurteaux, C., and Lazdunski, M. (1997) TASK, a human background K+ channel to sense external pH variations near physiological pH. *EMBO J* **16**, 5464-5471
3. Olschewski, A., Li, Y., Tang, B., Hanze, J., Eul, B., Bohle, R. M., Wilhelm, J., Morty, R. E., Brau, M. E., Weir, E. K., Kwapiszewska, G., Klepetko, W., Seeger, W., and Olschewski, H. (2006) Impact of TASK-1 in human pulmonary artery smooth muscle cells. *Circ Res* **98**, 1072-1080
4. Olschewski, A. (2010) Targeting TASK-1 channels as a therapeutic approach. *Adv Exp Med Biol* **661**, 459-473
5. Patel, A. J., Honore, E., Lesage, F., Fink, M., Romey, G., and Lazdunski, M. (1999) Inhalational anesthetics activate two-pore-domain background K+ channels. *Nat Neurosci* **2**, 422-426
6. Wilke, B. U., Lindner, M., Greifenberg, L., Albus, A., Kronimus, Y., Bunemann, M., Leitner, M. G., and Oliver, D. (2014) Diacylglycerol mediates regulation of TASK potassium channels by Gq-coupled receptors. *Nat Commun* **5**, 5540
7. Streit, A. K., Netter, M. F., Kempf, F., Walecki, M., Rinne, S., Bollepalli, M. K., Preisig-Muller, R., Renigunta, V., Daut, J., Baukrowitz, T., Sansom, M. S., Stansfeld, P. J., and Decher, N. (2011) A specific two-pore domain potassium channel blocker defines the structure of the TASK-1 open pore. *J Biol Chem* **286**, 13977-13984
8. O'Donohoe, P. B., Huskens, N., Turner, P. J., Pandit, J. J., and Buckler, K. J. (2018) A1899, PK-THPP, ML365, and Doxapram inhibit endogenous TASK channels and excite calcium signaling in carotid body type-1 cells. *Physiol Rep* **6**, e13876
9. Czirjak, G., and Enyedi, P. (2002) Formation of functional heterodimers between the TASK-1 and TASK-3 two-pore domain potassium channel subunits. *J Biol Chem* **277**, 5426-5432
10. Barel, O., Shalev, S. A., Ofir, R., Cohen, A., Zlotogora, J., Shorer, Z., Mazor, G., Finer, G., Khateeb, S., Zilberberg, N., and Birk, O. S. (2008) Maternally inherited Birk Barel mental retardation dysmorphism syndrome caused by a mutation in the genomically imprinted potassium channel KCNK9. *American journal of human genetics* **83**, 193-199
11. Miller, A. N., and Long, S. B. (2012) Crystal structure of the human two-pore domain potassium channel K2P1. *Science* **335**, 432-436
12. Brohawn, S. G., del Marmol, J., and MacKinnon, R. (2012) Crystal structure of the human K2P TRAAK, a lipid- and mechano-sensitive K+ ion channel. *Science* **335**, 436-441
13. Dong, Y. Y., Pike, A. C., Mackenzie, A., McClenaghan, C., Aryal, P., Dong, L., Quigley, A., Grieben, M., Goubin, S., Mukhopadhyay, S., Ruda, G. F., Clausen, M. V., Cao, L., Brennan, P. E., Burgess-Brown, N. A., Sansom, M. S., Tucker, S. J., and Carpenter, E. P. (2015) K2P channel gating mechanisms revealed by structures of TREK-2 and a complex with Prozac. *Science* **347**, 1256-1259

14. Lolicato, M., Arrigoni, C., Mori, T., Sekioka, Y., Bryant, C., Clark, K. A., and Minor, D. L., Jr. (2017) K2P2.1 (TREK-1)-activator complexes reveal a cryptic selectivity filter binding site. *Nature* **547**, 364-368
15. Sediva, M., Lassuthova, P., Zamecnik, J., Sedlackova, L., Seeman, P., and Haberlova, J. (2019) Novel variant in the KCNK9 gene in a girl with Birk Barel syndrome. *Eur J Med Genet*
16. Sitsel, O., Wang, K., Liu, X., and Gourdon, P. (2016) Crystallization of P-type ATPases by the High Lipid-Detergent (HiLiDe) Method. *Methods Mol Biol* **1377**, 413-420

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