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online data

## SUPPLEMENTARY ONLINE DATA

# Reorientation of the first signal-anchor sequence during potassium channel biogenesis at the Sec61 complex

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## EXPERIMENTAL

### Immunofluorescence microscopy

HeLaM cells grown on glass coverslips were transfected with plasmids encoding OPG–TASK75, OPG–TASK158 or the full-length version of the protein, using Lipofectamine™ 2000 in accordance with the manufacturer's instructions. Cells were fixed and permeabilized in −20 °C methanol for 5 min. Anti-opsin antibody was used to label OPG–TASK polypeptides, and anti-calnexin antibody (Sigma) was used as a marker for the endoplasmic reticulum. Alexa Fluor® 488- and 594-conjugated secondary antibodies were purchased from Jackson ImmunoResearch and DNA was stained using DAPI. Coverslips were mounted using Prolong Gold (Life Technologies) and fluorescence was visualized using a widefield Olympus BX-60 microscope with a ×60 1.40 N.A. (numerical aperture) PlanApo objective and a CoolSnap ES camera (Roper Scientific), with images captured using MetaVue software.



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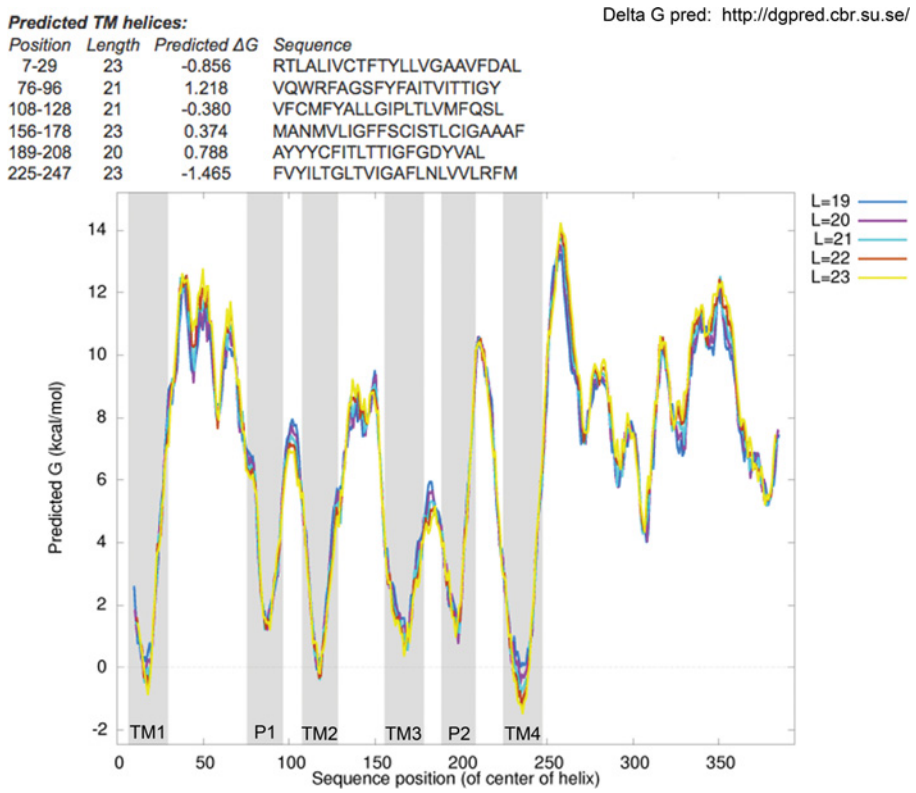


**Figure S1 Transmembrane domain prediction of TASK-1**

The human TASK-1 protein sequence was entered into the ΔG prediction server version 1.0 online at http://dgpred.cbr.su.se/. This predicted not only the four transmembrane domains (TM1–TM4), but also predicted the pore loops P1 and P2 as transmembrane domains. 1 kcal = 4.184 kJ.

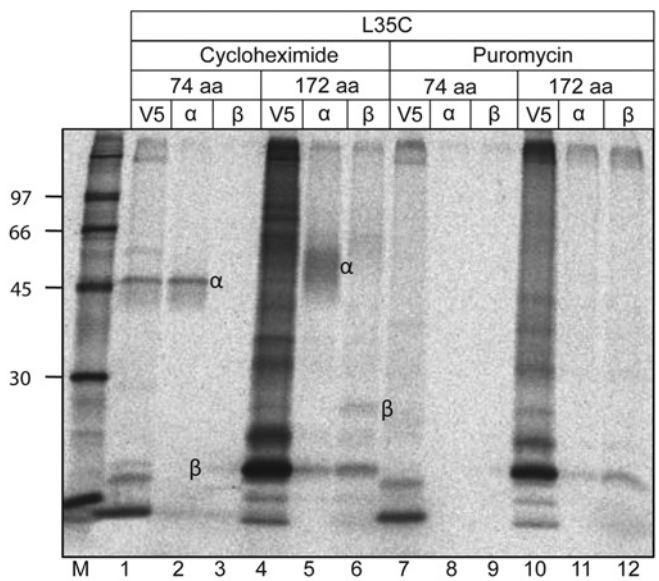


**Figure S2 Cross-linking following puromycin release**

TASK-1 truncations E60 and N158 lacking stop codons, both with the single cysteine mutation L35C and V5 tag were translated *in vitro*. Samples were then treated with either cycloheximide (as in all cross-linking in the present study) or puromycin (to release the nascent chain and dissociate the ribosome). BMH cross-linking was then carried out, and samples were subjected to immunoprecipitation with antibodies against the V5 tag (V5), Sec61$\alpha$ ($\alpha$) or Sec61$\beta$ ($\beta$). Adducts with Sec61$\alpha$ and Sec61$\beta$ are indicated. Molecular masses are indicated in kDa.

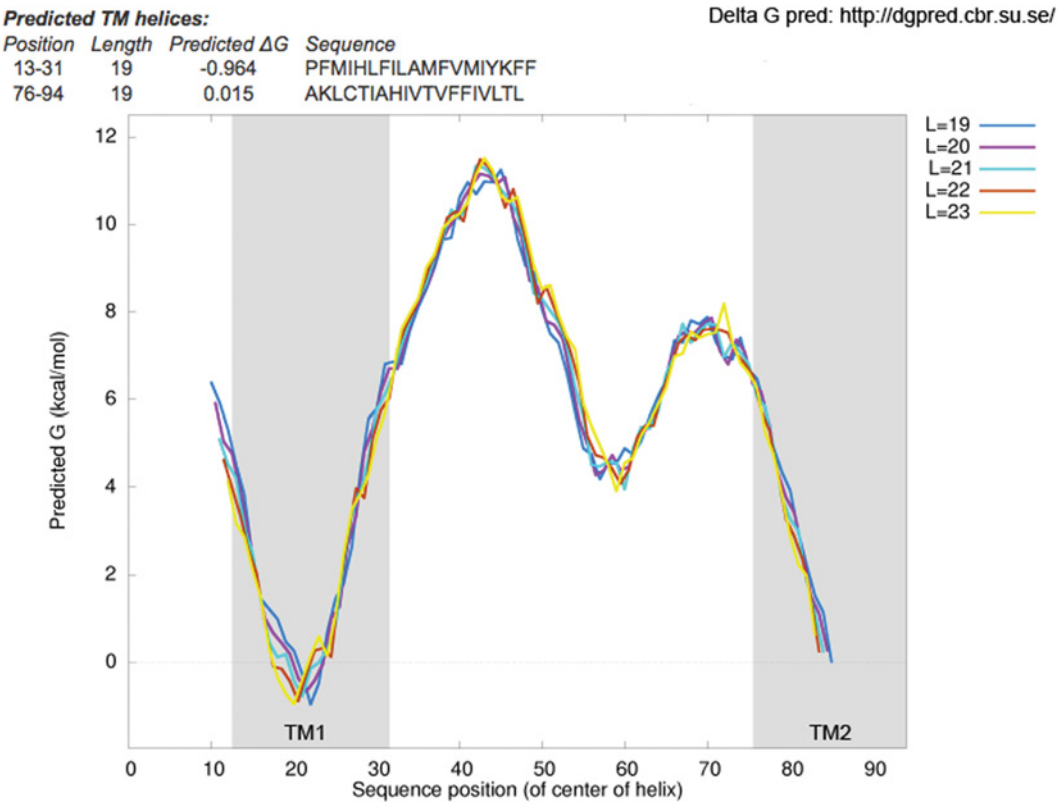


**Figure S3 Transmembrane domain prediction of Kcv**

The ΔG prediction server version 1.0 (online at http://dgpred.cbr.su.se/) was used to predict the location of the two transmembrane domains in Kcv. 1 kcal = 4.184 kJ.

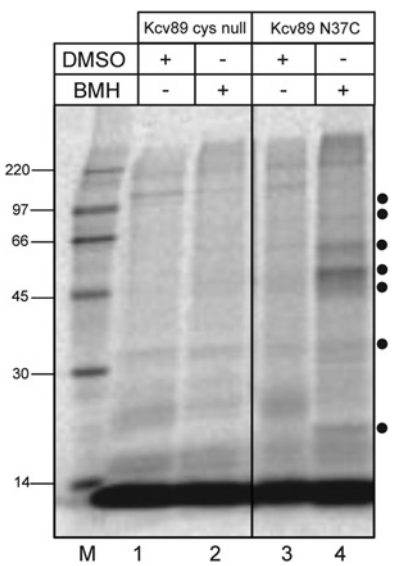


**Figure S4 Cysteine-dependent adducts with Kcv membrane integration intermediates**

The N-terminal 75 residues of a Cys-null form of Kcv (lanes 1 and 2), or a version with a single cysteine probe located at residue 37 (N37C, lanes 3 and 4), were synthesized using truncated mRNAs that incorporated a 14-residue V5 epitope tag at their C-termini, but lacked a stop codon. The resulting ribosome-bound peptidyl-tRNAs generated membrane-integration intermediates in the presence of ER-derived microsomes and, following isolation, these were treated with DMSO (lanes 1 and 3) or BMH (lanes 2 and 4) as indicated. A number of discrete BMH-dependent cross-linking products were observed with the N37C, but not the Cys-null variant (lane 4, filled circles). Molecular masses are indicated in kDa.

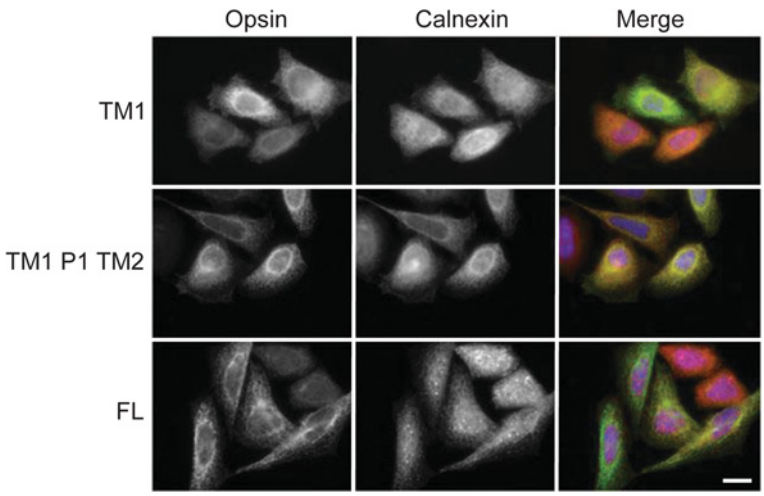


**Figure S5 TASK-1-derived products localize to the ER**

(**A**) HeLaM cells transiently expressing OPG-tagged fragments or full-length (FL) TASK-1 were methanol-fixed cells and stained with antibodies against the OPG tag and calnexin. Scale bars, 20 $\mu$m.

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