Mechanism of the Spontaneous and Directional Membrane Insertion of a 2-Transmembrane Ion Channel.

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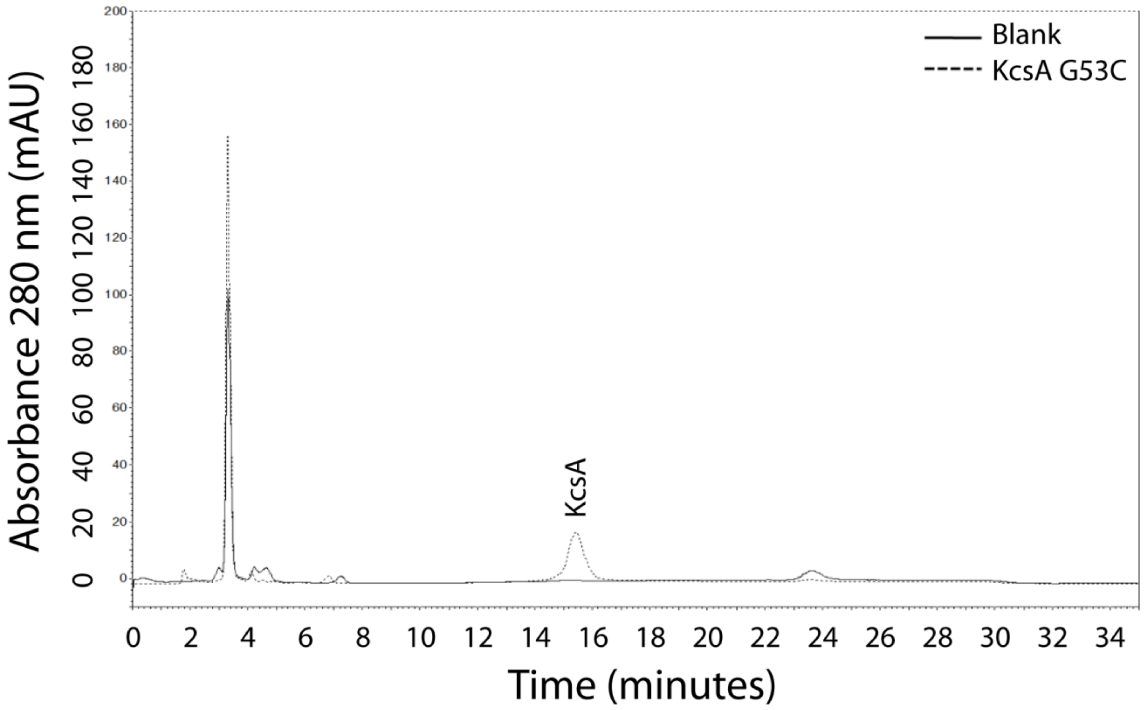
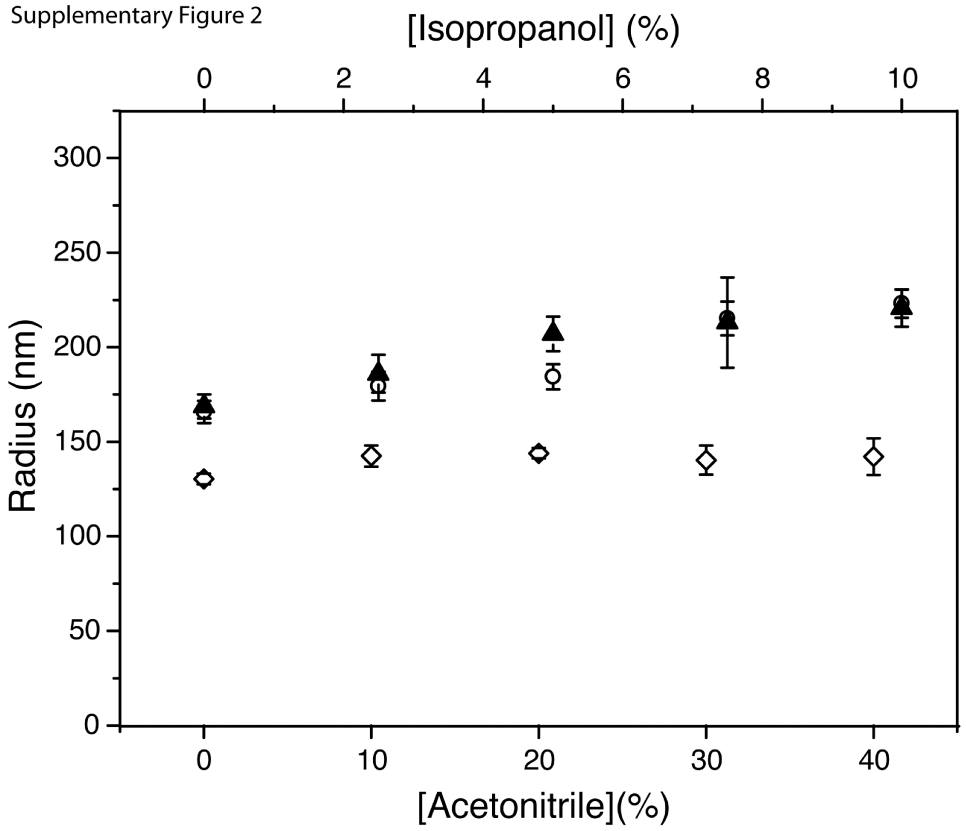
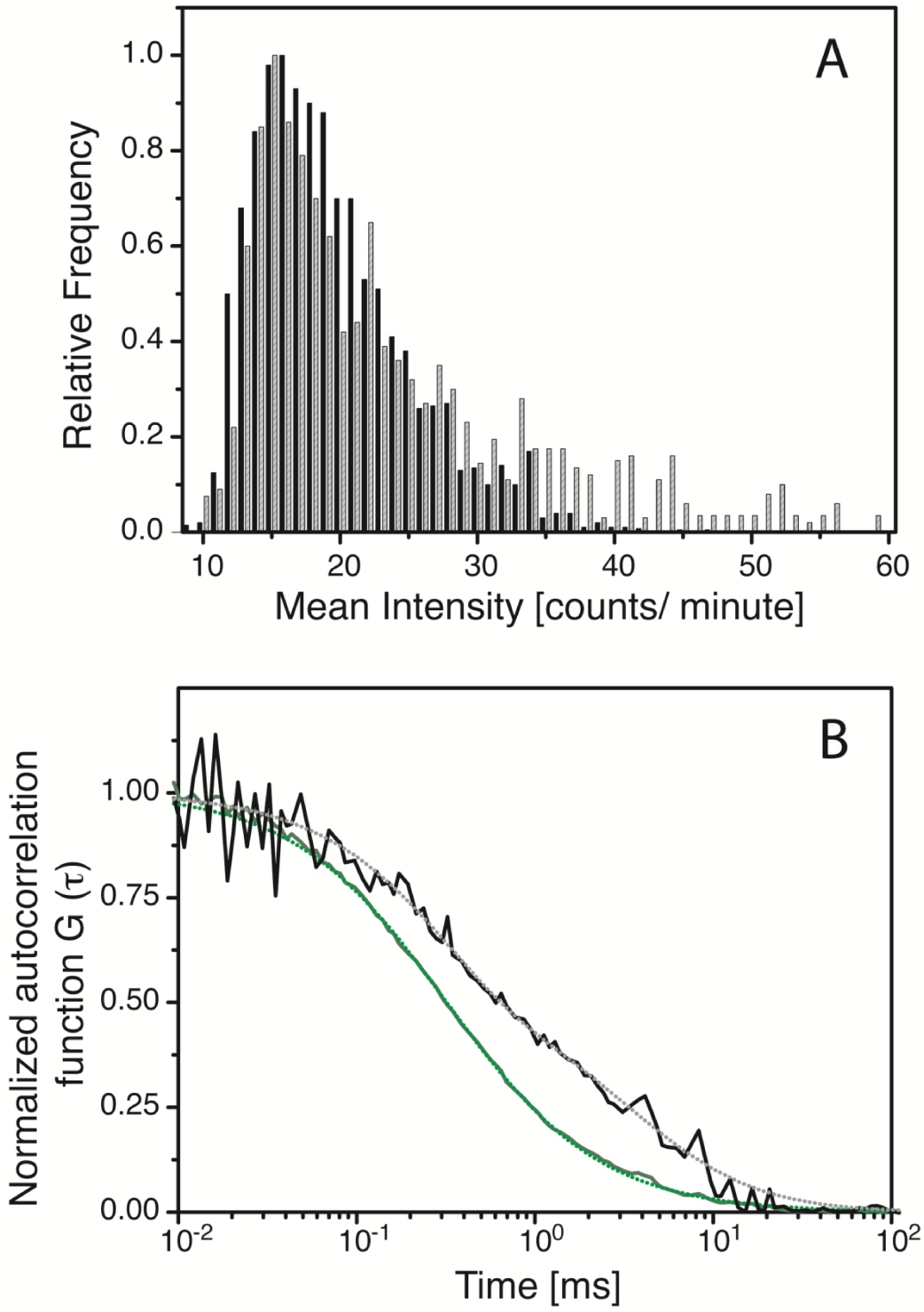
Supporting Information:



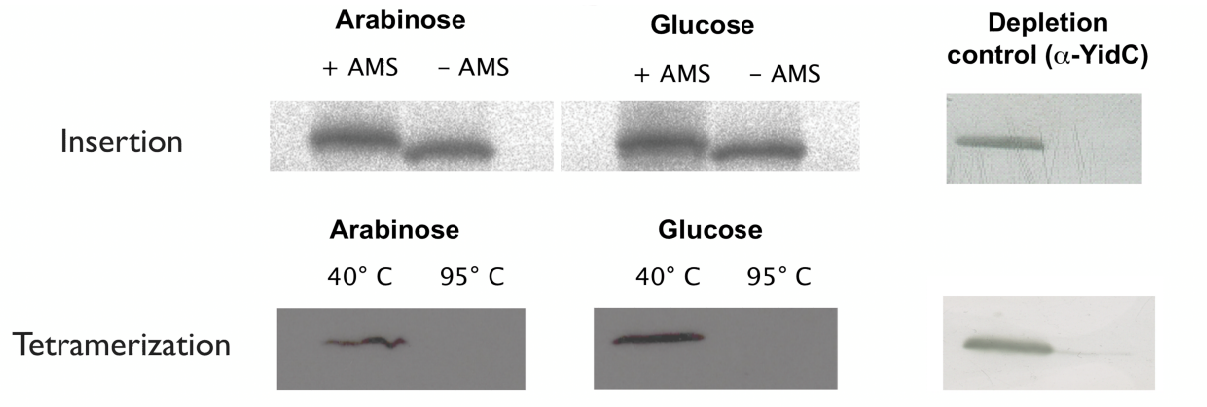
Suppl. figure 1: HPLC chromatogram of KcsA-G53C. Acetonitrile (ACN), was used to ensure complete monomerization and unfolding of detergent-solubilized KcsA. After resuspension and solubilisation of acetone precipitated KcsA in 60 % ACN, a water:ACN gradient chromatographic run was performed using a Eurosil Bioselect 300-10 C4 (Knauer, Berlin, Germany) column. A blank sample containing identical solvent concentrations was run as a control. The test sample shows a distinct peak for the KcsA protein at approximately 15.5 minutes running time and migrates as a monomer on a SDS PAGE gel.



Suppl. figure 2: DOPC liposome stability was measured by dynamic light scattering (DLS). The influence of acetonitrile (ACN) on liposome size and stability was measured up to 40 % ACN (open diamonds). The influence of isopropanol on size and integrity of liposomes was measured in absence (open circles) and presence of 3 M Guanidinium hydrochloride (triangles).



Suppl. figure 3: Histogram of Atto520 dye intensities and dye-labeled KcsA. As the distribution of the protein is similar to the dye (black columns), a monomeric and non-aggregated protein species can be assumed (grey columns). Aggregates occur only to a minor extent. (B): Autocorrelation function of Atto520 dye (green) and dye labeled KcsA-G53C (grey) measured by FCS (see materials and methods).



Suppl. figure 4: *In vivo* derivatization of KcsA-G53C with AMS. AMS is an inner membrane impermeable chemical that can traverse the outer membrane of *E. coli*. It reacts with thiol groups of accessible proteins in the outer membrane and the periplasm as well as accessible loops of inner membrane proteins such as KcsA (the periplasm accessible loop between the two transmembrane helices contains the Glycine-53 to Cysteine mutation). Upon insertion of KcsA and derivatization with AMS, its molecular weight is increased thus leading to a slower migration behavior on SDS-PAGE gels. The figure shows immunoblots against the C-terminal Hexahistidine- tagged KcsA. We used the *E. coli* YidC-depletion strain MK6 with an arabinose-promoter controlled expression of the insertase YidC. The presence of arabinose leads to the expression of YidC while the presence of glucose strongly represses the expression of YidC, leading to a depletion (right panels, arabinose left and glucose right). In presence of YidC, the addition of AMS leads to a shift in apparent size of KcsA (panel left, left). Under YidC-depletion conditions, the insertion of KcsA is not reduced as visualized on the gel (middle panel, left). Also tertramerization is YidC-independent; KcsA runs as a tetramer in SDS-PAGE gels when it is not heated (40° C), while the tetramer falls apart when heated (95° C). The absence of YidC does not reduce the amount of tetramer (middle panel, left) as compared to the presence of YidC (left panel, left).

AMS, 4-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid