



Supplementary information, Fig. S4 Single-particle cryo-EM analysis of nucleotide-free MlaFEDB in nanodiscs. **a**, Representative cryo-EM image and 2D class averages of cryo-EM particle images of nucleotide-free MlaFEDB in nanodiscs. **b**, Angular distribution of particle images included in the final 3D reconstruction. **c** and **d**, Local resolution of the final cryo-EM map of nucleotide-free MlaFEDB focused on the MlaD subunits or MlaFEB subunits, respectively. **e**, Fourier shell correlation (FSC) curves: gold-standard FSC curve for overall structure (blue), MlaD subunits (orange) and MlaFEB subunits (green) with indicated resolution at FSC=0.143, **f** and **g**, FSC curve of MlaD subunits (orange) and MlaFEB subunits between the atomic model and the final map with indicated resolution at FSC=0.5 (black); FSC curve between half map 1 (red) or half map 2 (green) and the atomic model refined against half map 1. **h**, Cryo-EM densities superimposed with the atomic model for individual TM helices in the nucleotide-free MlaE. **i**, Cryo-EM densities superimposed with the atomic model for selected regions of the NBDs (MlaF), demonstrating the clear separation of the β -strands and side chain densities.