An amphipathic Bax core dimer forms part of the apoptotic pore wall in the mitochondrial membrane

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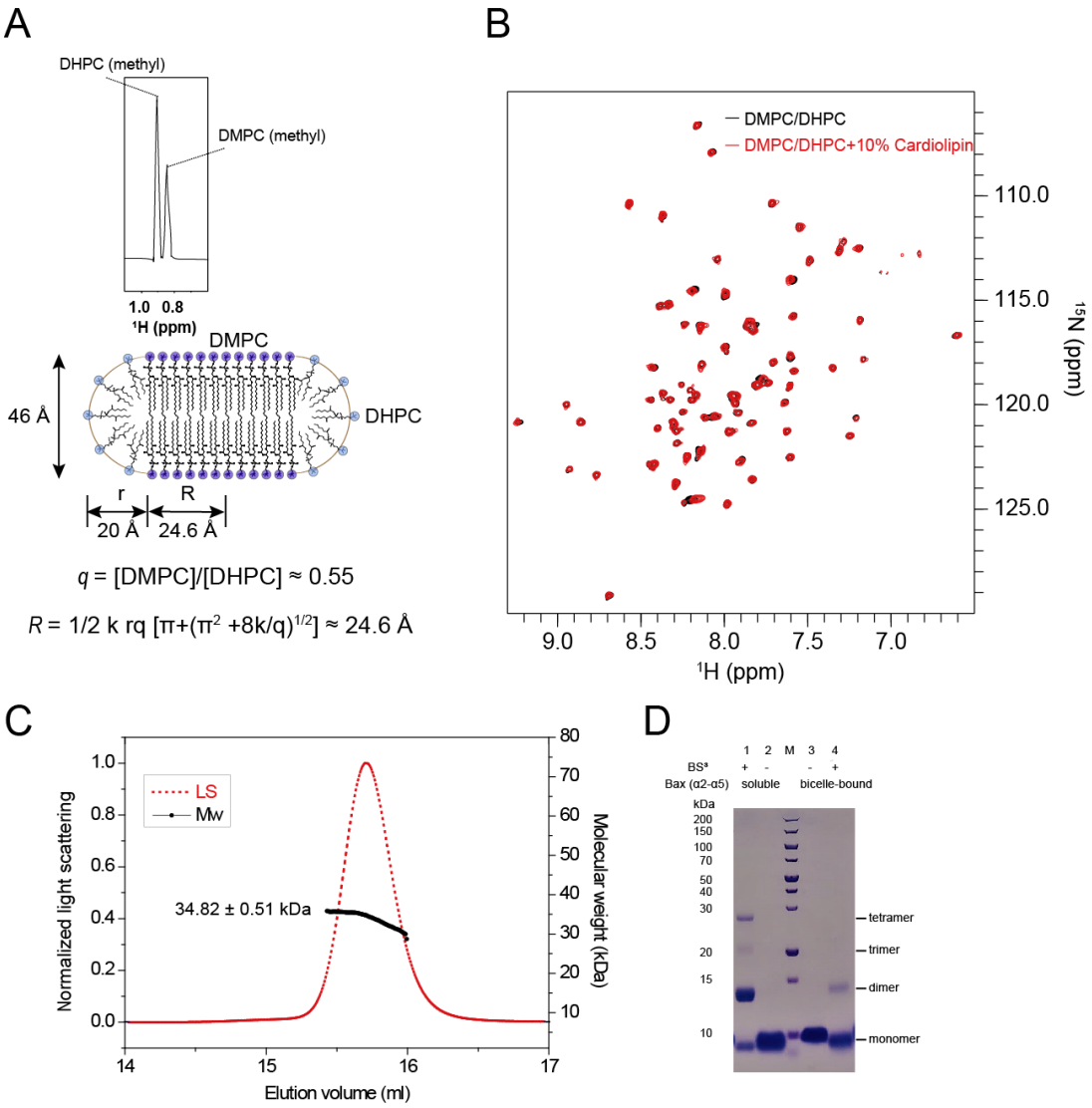


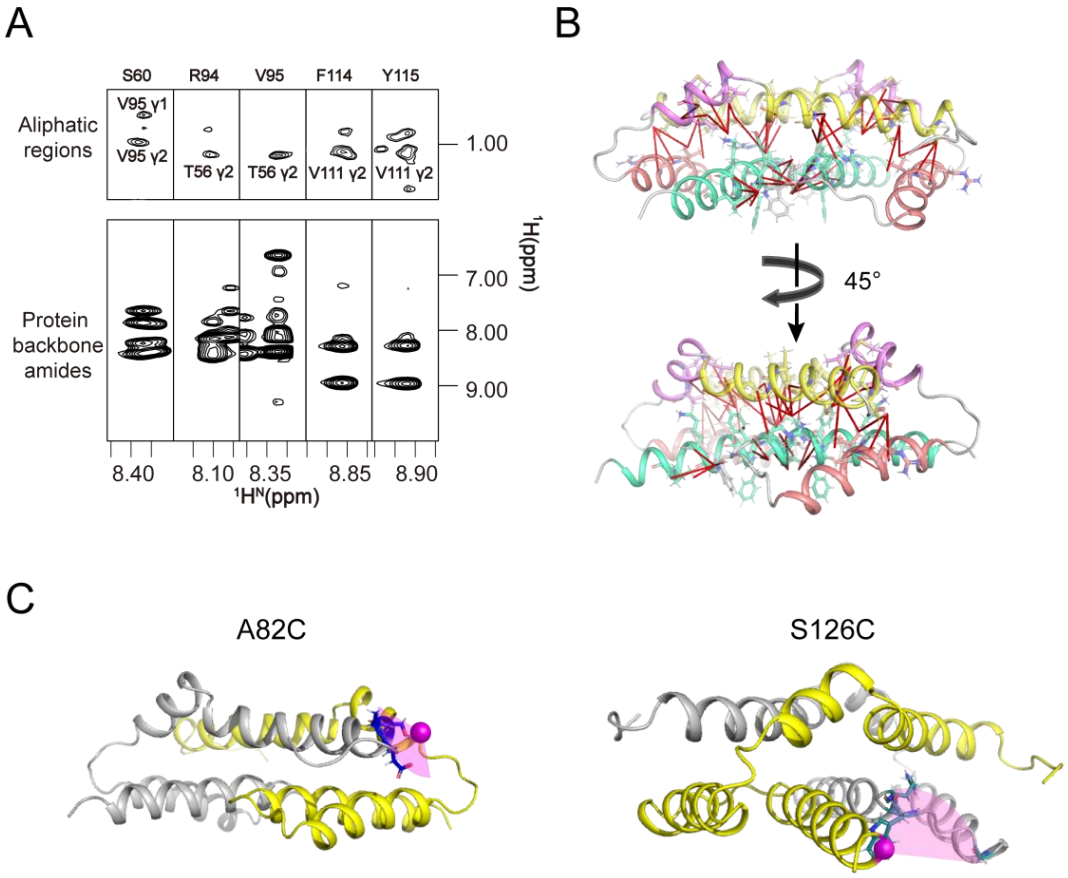
Appendix Figure S1, Related to Figure 1. Purification and preparation of Bax (α2-α5) in bicelles

(A) Characterization of the lipid bicelles used in NMR experiments. The molar ratio of DMPC to DHPC (q) in the bicelles determined from the one-dimensional ^{1}H spectrum on the left is ~ 0.55. The bicelle structure is illustrated with the planar lipid bilayer formed by DMPC and the micellar lipid rim formed by DHPC. The equation describes the radius of the planar region of the bicelle (R) as a function of the molar ratio of DMPC to DHPC (q), where k is the ratio of the head group area of DMPC to that of DHPC, and r is the radius of the rim. In this case, q = 0.55, k = 0.6, and r = 20 Å, thus, R = 24.6 Å.

(B) The superimposed 2D ^{1}H-^{15}N TROSY-HSQC spectra of DMPC/DHPC bicelle-bound Bax (α2-α5) without (black) or with (red) 10% cardiolipin recorded at 700 MHz.

(C) SEC-MALS analysis of the purified Bax (α2-α5) protein. The normalized light scattering (LS) and molecular weight (MW) are shown on the left and right axes, respectively. The black curve represents the calculated MW of the peak fractions of the eluted Bax (α2-α5) with the mean MW and standard deviation (SD) indicated. The theoretical MW for Bax (α2-α5) monomer is 9137.43 Da.

(D) Chemical crosslinking analysis of oligomerization of the bicelle-bound and soluble Bax (α2-α5) samples used in NMR experiments. The samples from the reactions with or without crosslinker BS^3 were analyzed by SDS-PAGE and Coomassie Blue staining. The molecular weights for the protein standards in lane M are indicated on the left side. The positions for the monomer and multimers are indicated on the right side.

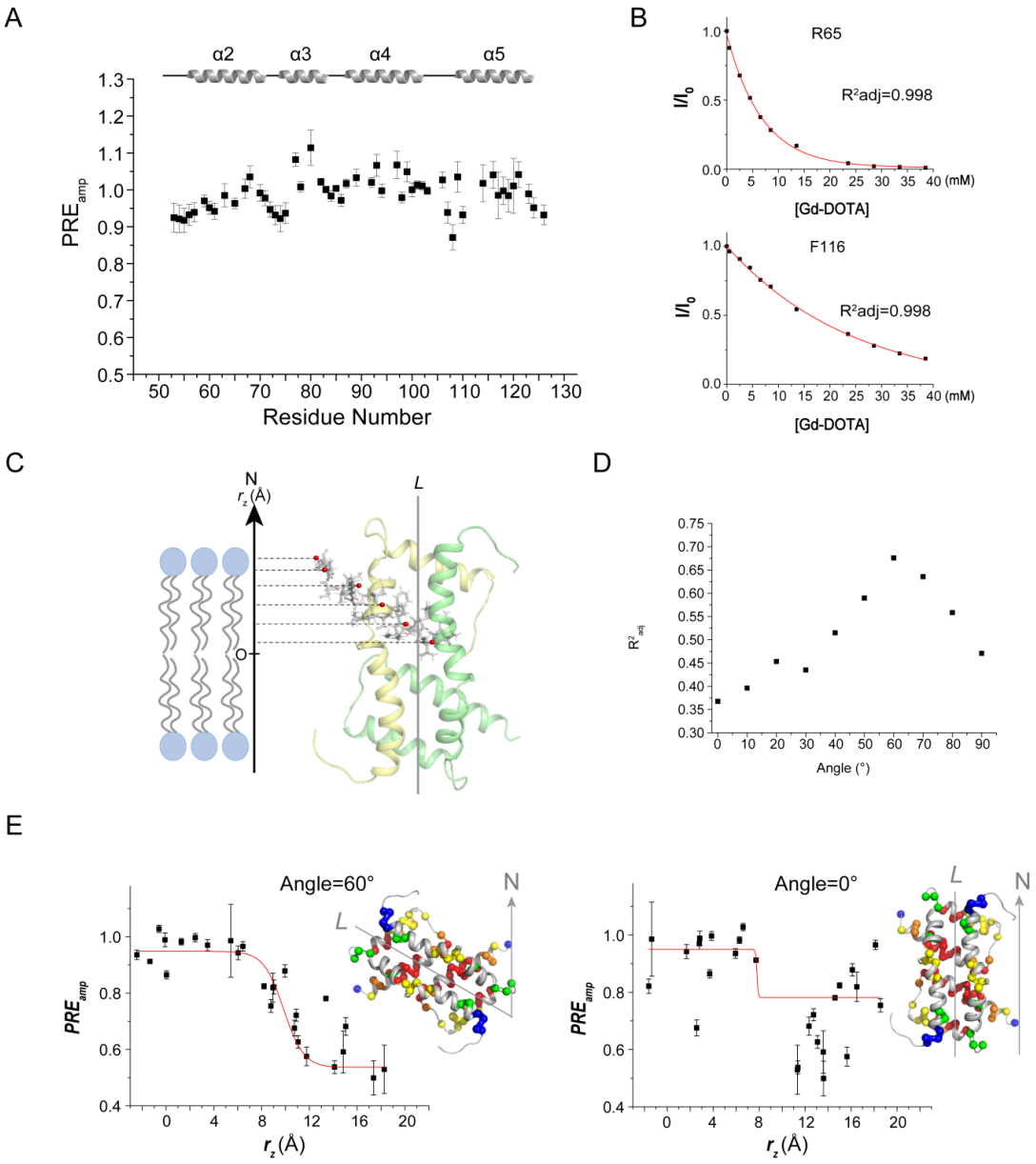


Appendix Figure S2, Related to Figure 2. Analysis of intermonomer restraints in Bax (α2-α5) in bicelles

(A) The representative NOE strips taken from 3D ^{15}N-edited NOESY-TROSY spectrum recorded at 900 MHz with 250-ms NOE mixing time. The use of mixed isotope-labeled monomers containing 50% [^{15}N-, ^{2}H]-labeled protein and 50% uniformly [^{13}C]-labeled protein permitted the assignment of intermonomer NOEs. The control spectrum from the sample with 100% [^{15}N, ^{2}H]-labeled protein (data not shown) demonstrated unambiguously that the crosspeaks in the aliphatic regions are the intermonomer NOEs between the backbone amide and the sidechain methyl protons.

(B) Sideview of the Bax (α2-α5) dimer structure with all intermonomer NOE-derived distance restraints used in structure determination shown as red lines.

(C) Interchain PRE analysis of Bax (α2-α5) in bicelles. Mapping the PREs onto the structure of Bax (α2-α5) in bicelles, showing the backbone amide protons (residue represented by a blue stick in each structure) in chain A (gray) with strong PREs to the nearby spin probe (represented by a magenta sphere in each structure) in chain B (yellow). The sample consisted of an ~1:1 mixture of [^{15}N]-labeled Bax (α2-α5) and [^{14}N]-labeled Bax (α2-α5) with a MTSL spin probe at A82C (left) or S126C (right).



Appendix Figure S3, Related to Figure 3. PRE analysis to determine the interaction of the Bax (α2-α5) dimer with the lipid bicelle

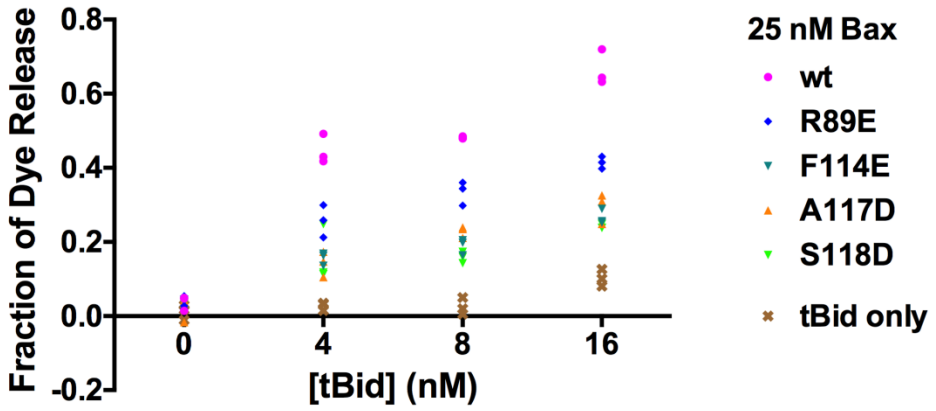
(A) Residue-specific PRE_{amp} of Bax (α2-α5) in bicelles derived from Gd-DOTA titration.

(B) The ratio of the ^{1}H-^{15}N TROSY-HSQC spectral peak intensities in the presence (I) and absence (I_0) of Gd-DOTA for residue R65 in the α2 helix or F116 in the α5 helix versus [Gd-DOTA].

(C) The NMR structure of the Bax (α2-α5) dimer with the longest axis (L) parallel to the lipid bicelle normal axis (N). The amide protons, for which PRE_{amp} has been determined, are shown as red spheres. The dashed lines point to the projected positions of these amide protons on the bilayer normal axis. The distances of these positions to an arbitrary point (O) on the axis are the r_z values used in the sigmoidal fitting shown in panel D.

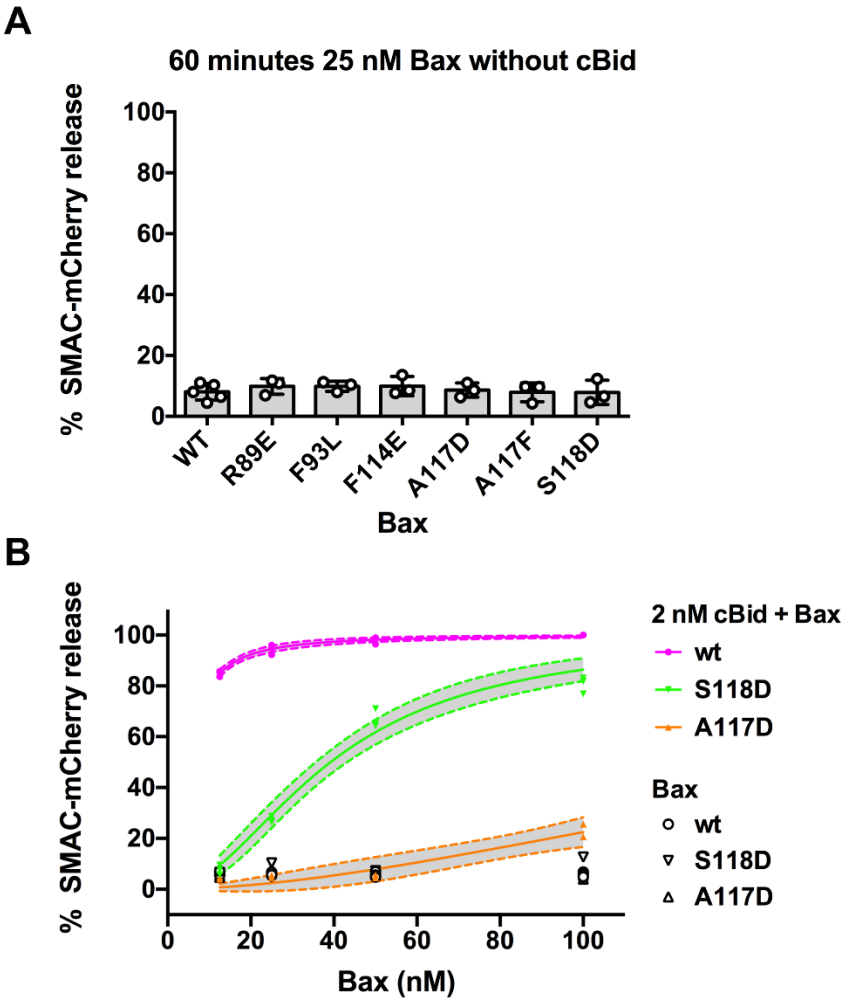
(D) R^2_{adj} versus Angle plot. The quality of the fitting (R^2_{adj}) of the PRE_{amp} (r_Z) data using the symmetric sigmoidal function (Eq. 2) increases as the Bax (α2-α5) dimer rotates closer to the correct orientation relative to the bilayer, which is measured by the Angle between the L and N axes.

(E) PRE_{amp} versus r_Z plots. The structures on the right illustrate two orientations of the Bax (α2-α5) dimer (L) relative to the bilayer normal (N), which result in different r_z values for the amide protons, and hence, affect the fitting of the PRE_{amp} vs. r_z data using the symmetric sigmoidal function (Eq. 2) as shown by the red fitting curves in the PRE_{amp} (r_z) plots. The best fitting is achieved at the angle of 60^o, whereas the worst fitting is achieved at the angle of 0^o.



Appendix Figure S4, Related to Figure EV4. The effect of tBid concentrations on the fluorescent dye release of Bax mutants

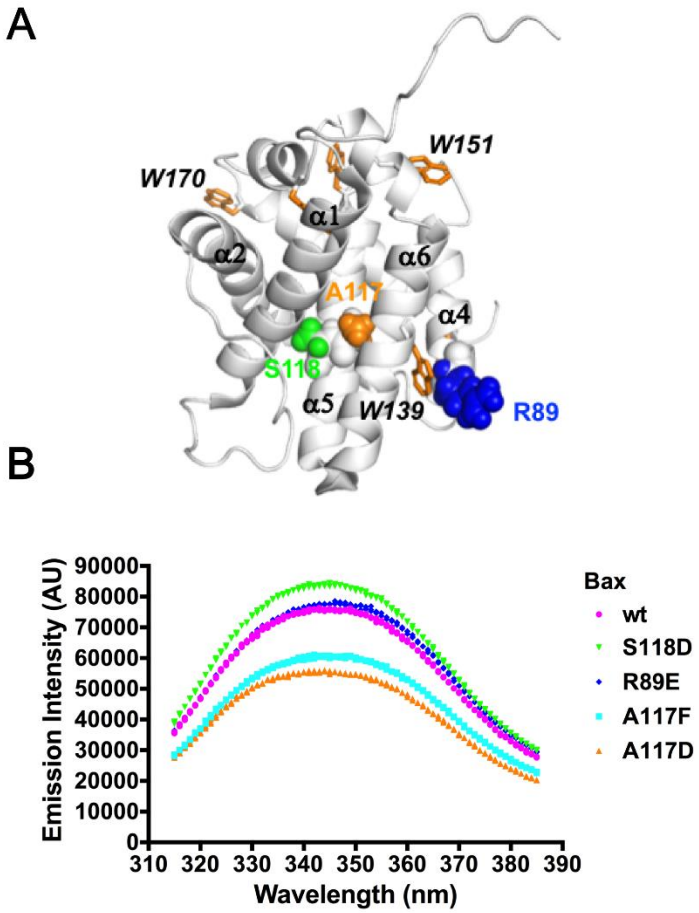
The fluorescent dye release from mitochondrion-mimic liposomes by 25 nM wt or mutant Bax in the presence or absence of 4, 8 or 16 nM tBid as indicated was measured after 480-min incubation. The fraction of dye release was normalized to that by detergent and shown as means (symbols) ± SD (error bars) from three independent experiments.



Appendix Figure S5, Related to Figure 4. Mitochondrial permeabilization activity of Bax mutants in the absence of cBid or at higher concentrations.

(A) Digitonin permeabilized BMK Bax$^{-/-}$ Bak$^{-/-}$ cells expressing SMAC-mCherry in the mitochondria intermembrane space (50 µL, 500,000 total cells) were incubated with 25 nM of Bax for 60 minutes at 37 °C in a 96 well plate. The samples were centrifuged for 10 minutes and separated into supernatant and pellet fractions. SMAC-mCherry release was calculated as the fraction of total (supernatant + pellet) mCherry fluorescence coming from the supernatant fraction. The data was normalized to the percent SMAC-mCherry release of WT Bax and cBid at 60 minutes. Each symbol represents the normalized SMAC-mCherry release for one single replicate (n=3 or more independent replicates). The bars indicate the average % SMAC-mCherry release for each condition.

(B) Identical (A) however the digitonin permeabilized cells were incubated with various concentrations of Bax with or without 2 nM cBid for 60 minutes at 37 °C. The data was normalized to the percent SMAC-mCherry release of 100 nM WT Bax and cBid at 60 minutes. Each symbol represents the normalized SMAC-mCherry release for one single replicate (n=3 or more independent replicates). Where applicable the data was fit with the [Agonist] vs. response -- Variable slope equation in Graphpad Prism 8.0.1. The dotted lines represent the 95% confidence interval of the fit.



Appendix Figure S6, Related to Figure 4. Tryptophan emission spectra of Bax proteins
(A) Bax protein structure. A ribbon diagram of the NMR structure of soluble Bax (PDB code: 1F16) is shown with some of the nine helices labeled. Some of the six Trp residues are visible and shown as orange sticks. R89 on the protein surface, and A117 and S118 in the protein core are shown as blue, orange and green spheres, respectively.
(B) Trp emission spectra of the indicated Bax proteins in solution. The data shown are means (lines) ± SD (error bars) from three technical repeats of one experiment.