Supplementary information
Computational design of Bax-inhibiting peptides

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The proteins of the Bcl-2 family play crucial roles in regulating apoptosis. It is divided into pro-survival and pro-apoptotic proteins that determine cellular fate. In particular, Bax is a crucial executor of apoptosis as its activation initiates the apoptotic phenotype. Hence, targeting this protein represents an attractive therapeutic approach, which can aid in regulating apoptotic signalling and potentially contribute to the development of novel therapies against cancer and neurodegenerative diseases. Here, we introduce a digital paradigm, which relies on rational design and computer simulations to develop and validate peptide-based agents that bind to Bax, thereby inhibiting its apoptotic properties. The peptides are rationally designed and optimized to bind to Bax starting from the crystal structures of affimers in complex with Bcl-2 proteins. Next, molecular dynamics simulations (MD) are employed to probe the stability of the Bax-peptide complexes and to estimate the binding free energies. The results show that the designed peptides bind with high affinity to Bax. Two of the designed peptides bind in the canonical hydrophobic groove (BH1 domain) of Bax and one peptide binds to the outside of the BH3 domain (α_2-helix). Notably, the peptides restrict the flexibility of the α_1-α_2 loop, modulating the bottom trigger site associated with toxicity. All in all, the results highlight the potential of these peptides as valuable tools for further exploration in modulating apoptotic pathways and set the structural foundation for a machine learning powered engine for peptide design.

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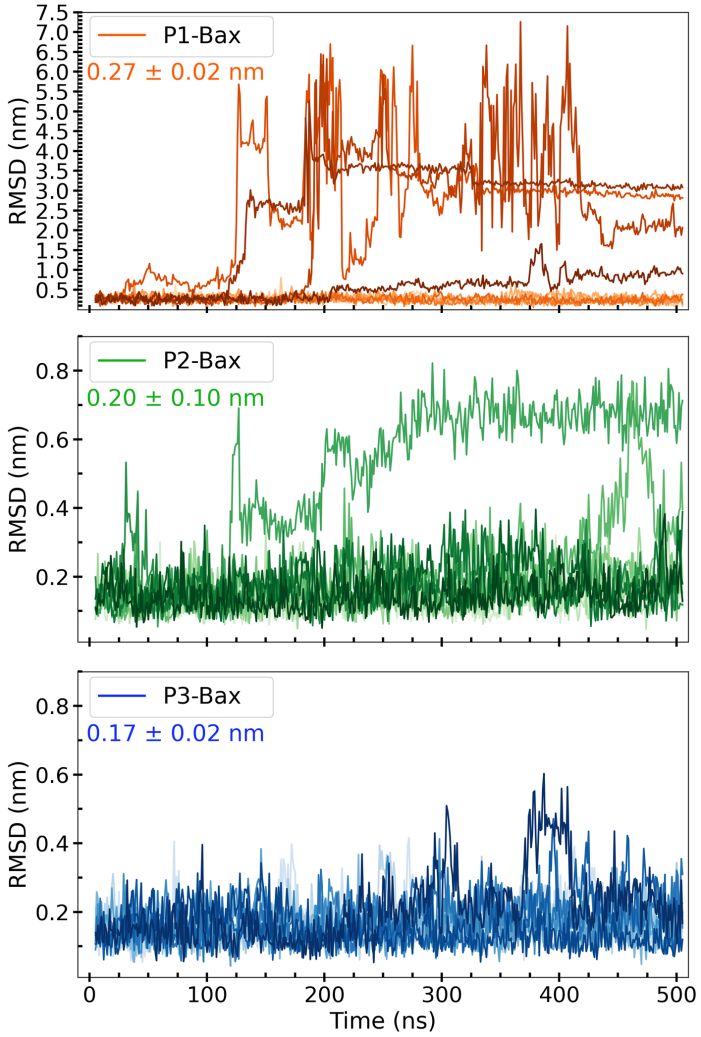

Fig. S1 Peptide stability. Shown are the time series of the displacement of the peptides with respect to Bax. First the structural alignment of the individual snapshots saved along the MD simulations is carried out on the C_α atoms of the secondary structure elements of Bax. Then for each MD snapshot the peptide C_α root-mean-square deviation (RMSD) is calculated as $\sqrt{\frac{1}{N_{ab}}\sum_{i=1}^{N_{ab}}(\mathbf{r_i}-\mathbf{r_i^{ref}})^2}$, where $\mathbf{r_i}$ and $\mathbf{r_i^{ref}}$ are the actual and reference coordinates, respectively, of the peptide C_α atom i. N_{ab} is the number of residues in the peptide. The high RMSD values for three of the ten P1 complexes indicate that the peptide detached from the Bax surface. Hence, for the analysis only the stable complexes are used. The values in the top left corners of the plots represent the average RMSDs and the errors represent the standard error of the mean calculated as the standard deviation of the average values over the independent stable runs.

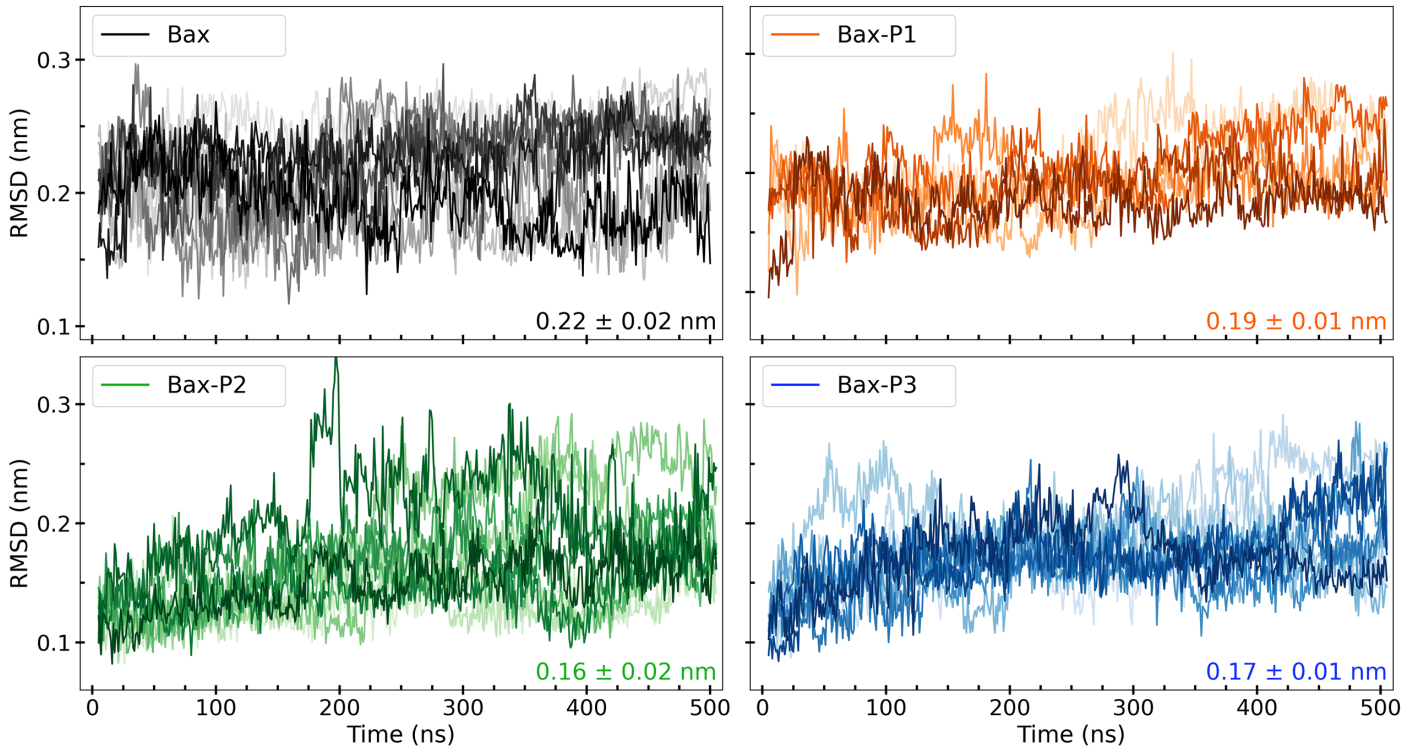

Fig. S2 Protein stability. Shown are the time series of the displacement of Bax with respect to the crystal structure. First the structural alignment of the individual snapshots saved along the MD simulations is carried out on the C_α atoms of the secondary structure elements of Bax. Then for each MD snapshot the peptide C_α root-mean-square deviation (RMSD) is calculated as $\sqrt{\frac{1}{N_{ab}}\sum_{i=1}^{N_{ab}}(\mathbf{r_i}-\mathbf{r_i^{ref}})^2}$, where $\mathbf{r_i}$ and $\mathbf{r_i^{ref}}$ are the actual and reference coordinates, respectively, of the protein C_α atom i. N_{ab} is the number of residues in Bax. The values in the lower right corners of the plots represent the average RMSDs and the errors represent the standard error of the mean calculated as the standard deviation of the average values over the independent runs (in which the peptides remain stably attached to Bax).

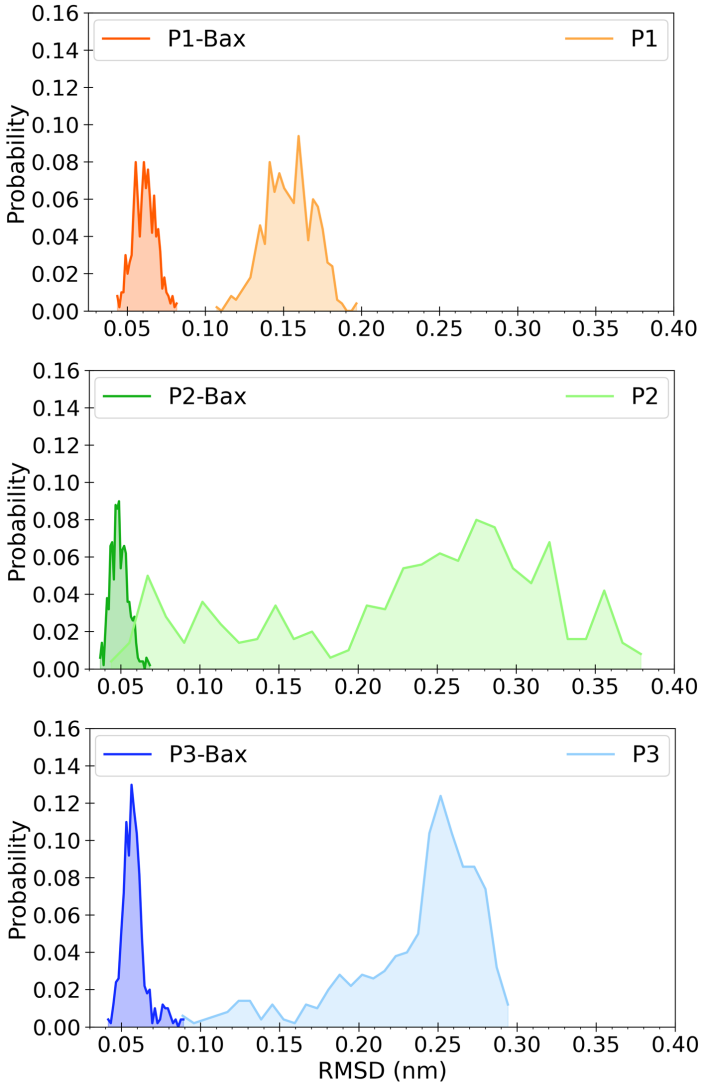

Fig. S3 Peptide conformations. Shown are the probability distributions of the peptide RMSDs in the bound (dark colors) and unbound states (light colors) relative to the bound state. The unbound peptide runs are started from the bound peptide conformations in absence of Bax. The analysis shows that the unbound peptides sample states that are different from the bound conformations, suggesting that Bax stabilizes specific intramolecular peptide bonds.

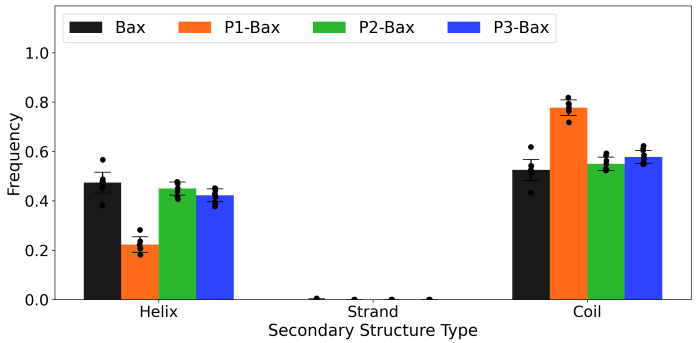

Fig. S4 Secondary structure of α_3. Shown are the normalized secondary structure assignments of α_3 and the α_3-α_4 loop in absence and presence of the peptides.

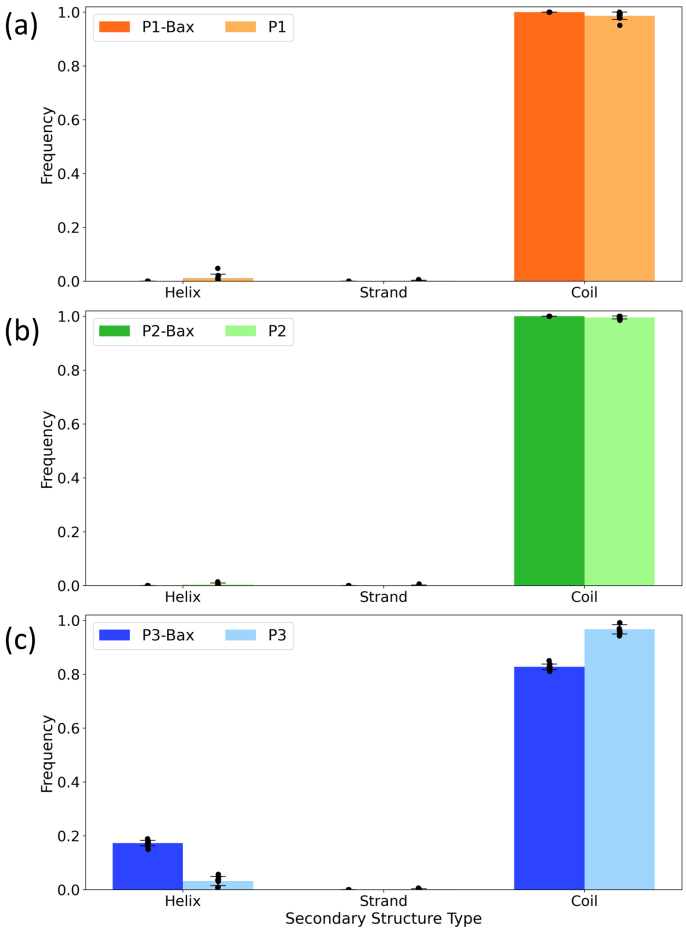

Fig. S5 Peptide secondary structure assignment. Shown are the normalized secondary structure assignments of the peptides in the bound and unbound states. The results show that the peptides adopt predominantly disordered structures, except for P3, which partially adopts and α-helical structure when bound to Bax.

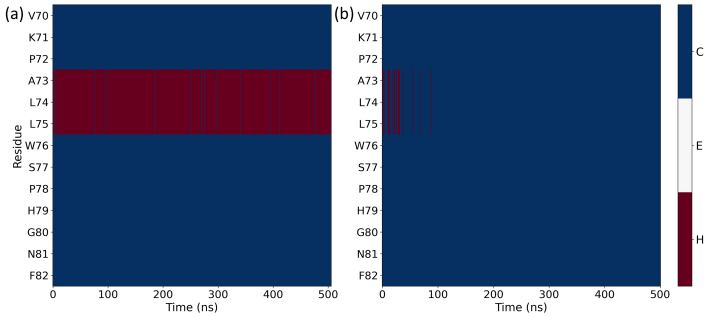

Fig. S6 Secondary structure of P3. Shown are the secondary structure time evolutions of P3 for the (a) bound and (b) unbound states, with C = coil, E = strand and H = helix.

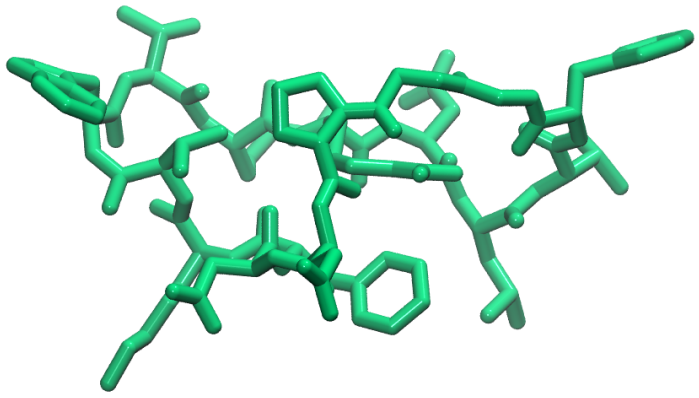

Fig. S7 P2 twisted boat conformation Shown is the twisted boat conformation of peptide P2.