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Supporting Information

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SUPPORTING INFORMATION

Sequence-Based Design of α/β-Peptide Foldamers that Mimic BH3 Domains

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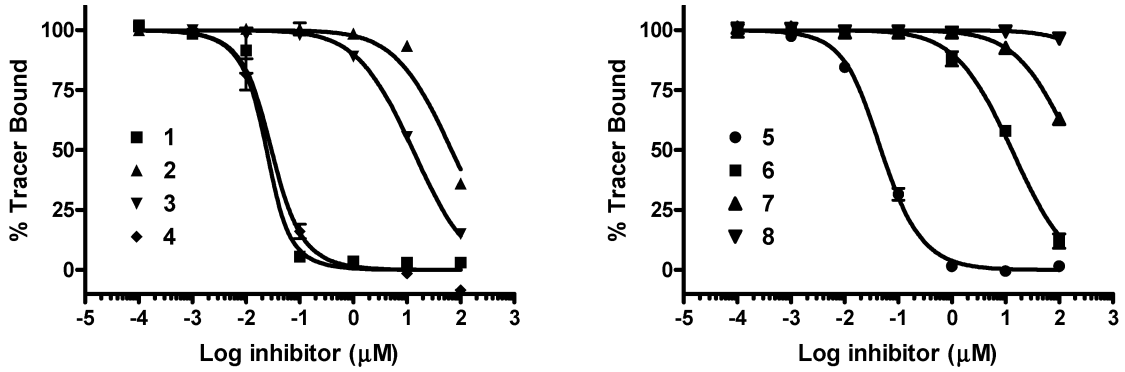

Figure S1. Bcl-x_L competition FP data for **1**-**8**. Curves result from fitting experimental data to analytical expressions for FP competitive binding.[S1]

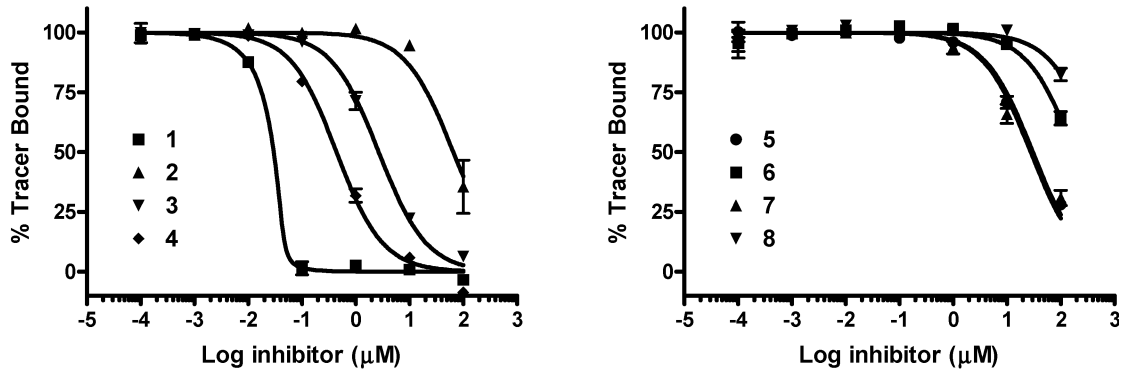

Figure S2. Mcl-1 competition FP data for **1**-**8**. Curves result from fitting experimental data to analytical expressions for FP competitive binding.[S1]

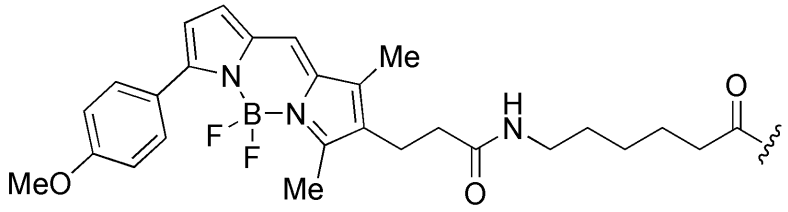

Figure S3. Structure the BODIPY-TMR fluorophore attached to the N-terminus of the fluorescently labeled analogues of **1**, **4**, and **5** used for direct binding FP experiments.

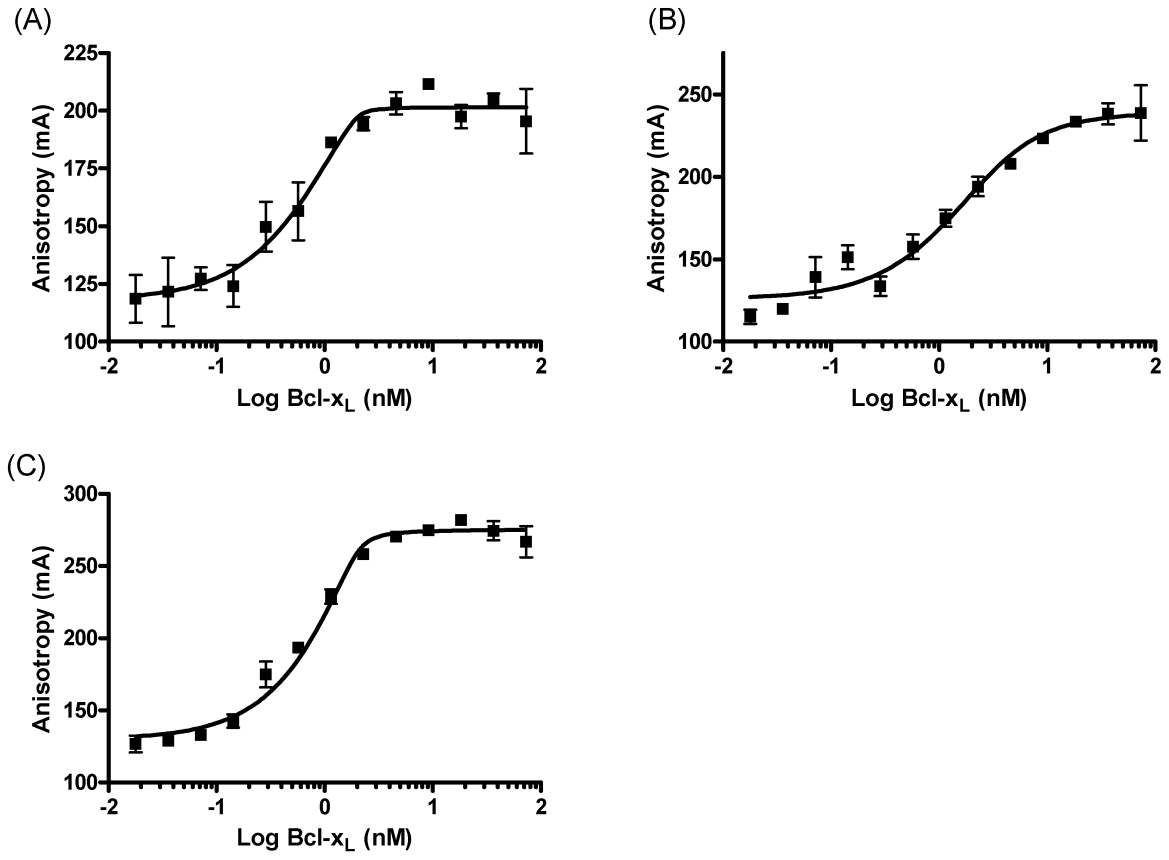

Figure S4. Bcl-x_L direct binding FP data for BODIPY-TMR-labeled analogues of (A) **1**, (B) **4**, and (C) **5**, each at 2 nM total tracer. Curves result from fitting the experimental data to analytical expressions for FP direct binding.[S2]

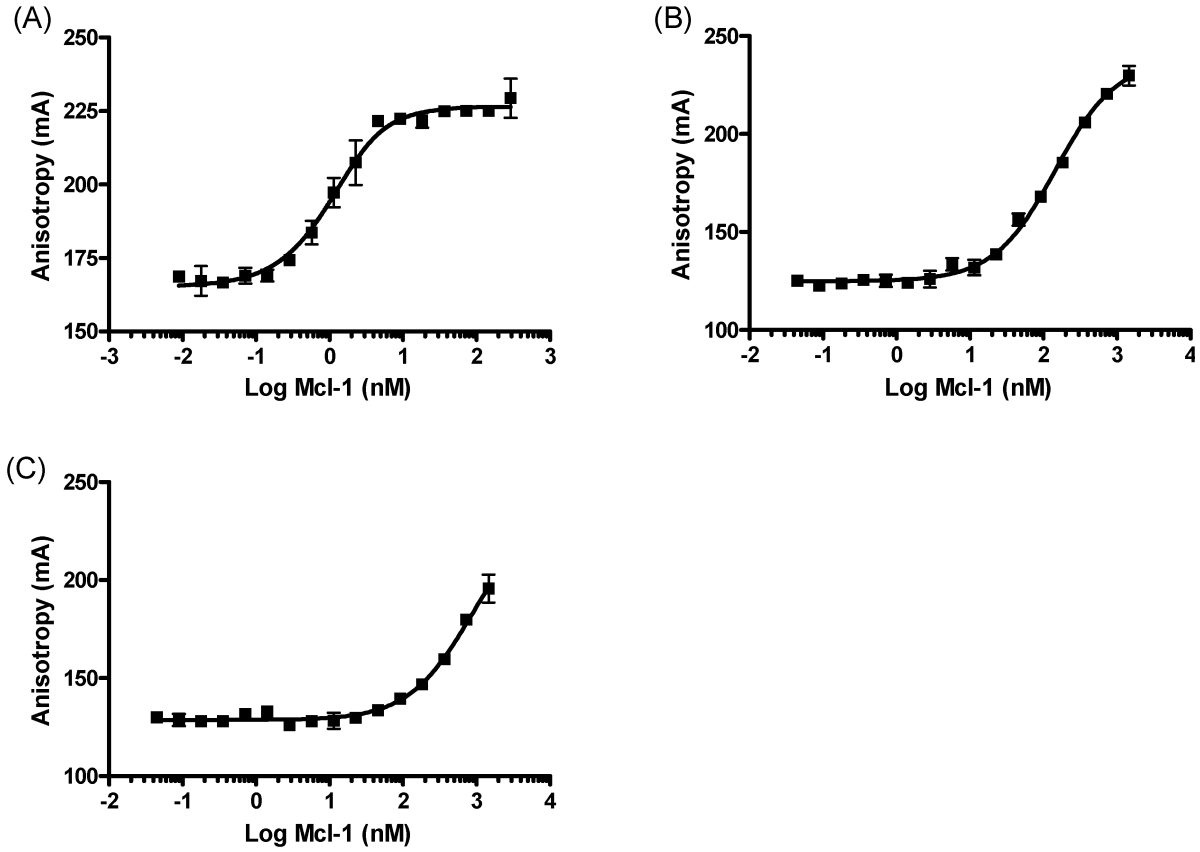

Figure S5. Mcl-1 direct binding FP data for BODIPY-TMR-labeled analogues of (A) **1**, (B) **4**, and (C) **5** at 2 nM, 20 nM, and 20 nM total tracer, respectively. Curves result from fitting the experimental data to analytical expressions for FP direct binding.[S2]

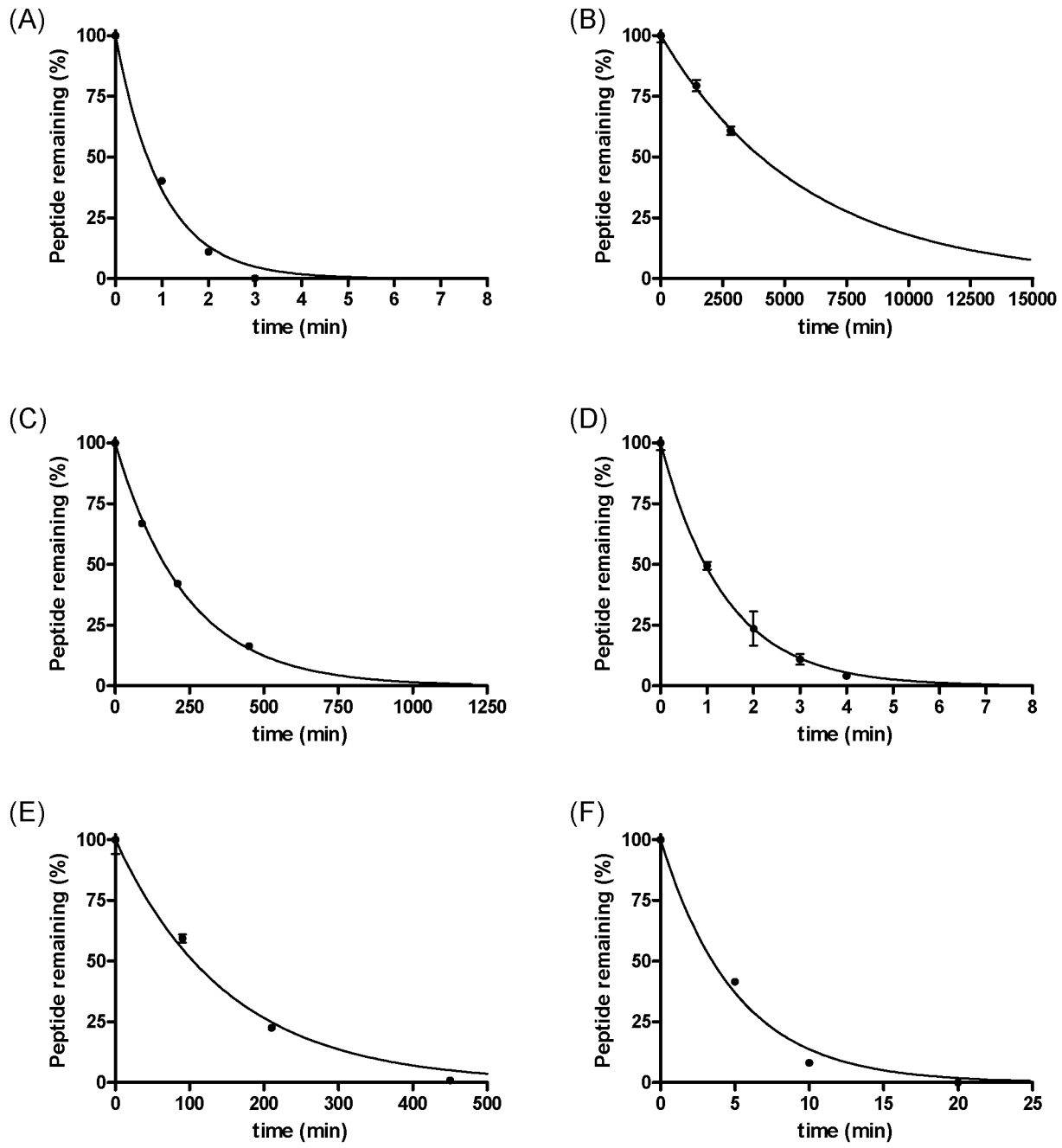

Figure S6. Time dependent proteolysis of α-peptide **1** and α/β-peptides **4** and **5**. (A) **1** , (B) **4**, and (C) **5** in the presence of 10 μg/mL proteinase K; (D) **1**, (E) **4**, and (F) **5** in the presence of 5 μg/mL pronase. Curves result from fitting the data to a simple exponential decay. Note the substantial differences among the x-axes. All reactions were carried out with 50 μM peptide in TBS.

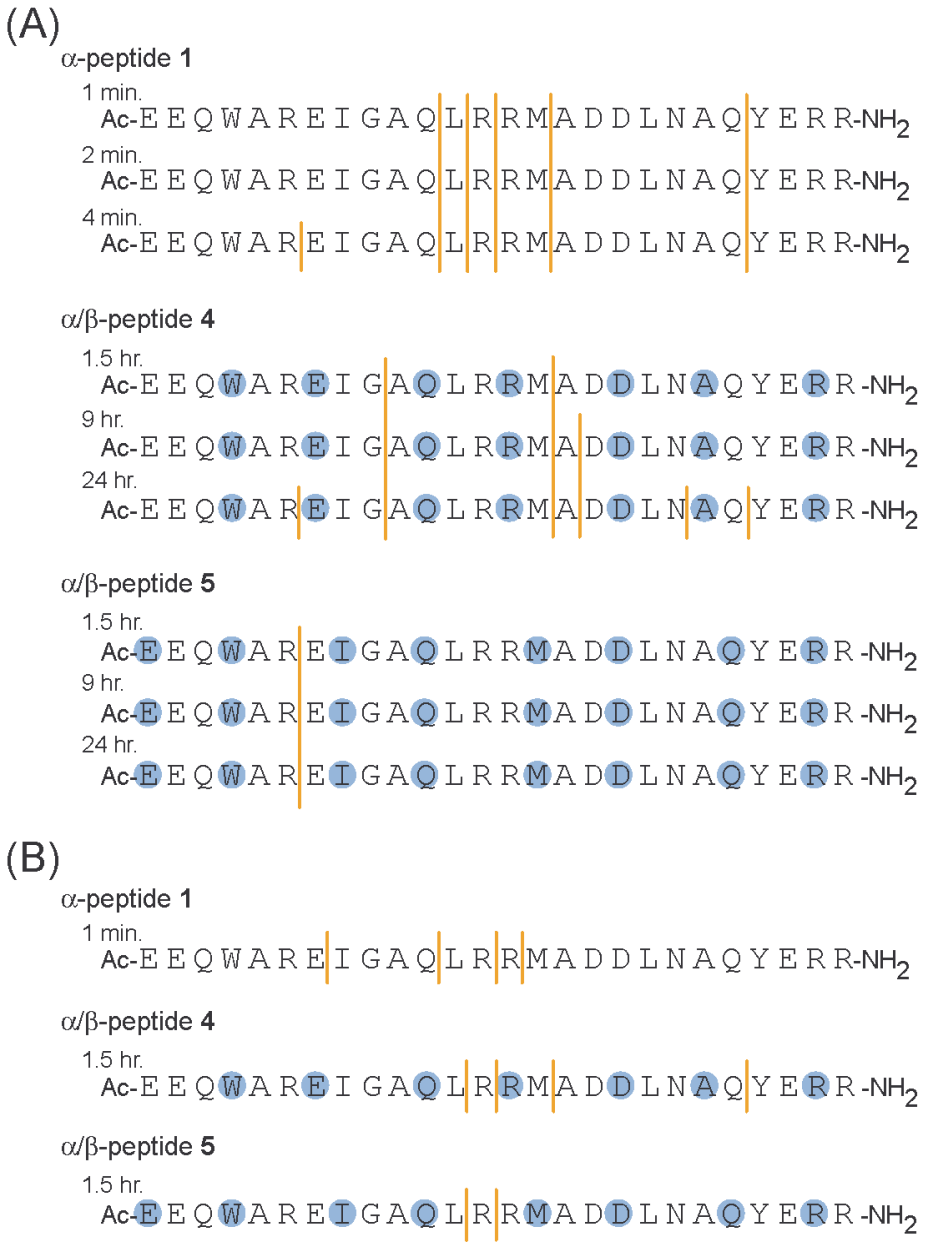

Figure S7. Proteolysis products observed for **1**, **4**, and **5** after treatment with (A) 10 μg/mL proteinase K and (B) 5 μg/mL pronase. Crude quenched reactions at the indicated time points were analyzed by MALDI-TOF-MS. The presence of a line between two residues indicates the detection in the mass spectrum of one product or both arising from cleavage at that point.

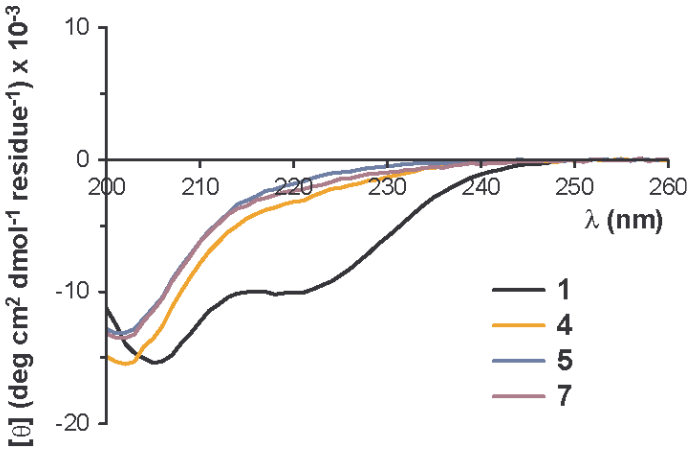

Figure S8. CD spectra of α-peptide **1** and α/β-peptides **4**, **5**, and **7** at 25 μM concentration in 10 mM phosphate, pH 7. Previous work has shown that $[\theta]_{206}$ is approximately -40,000 deg cm^2 dmol^{-1} res^{-1} for an α/β-peptide with an ααβαααβ backbone pattern in the fully folded helix bundle state[S3], suggesting that none of the Puma α/β-peptides has substantial helical content alone in solution.

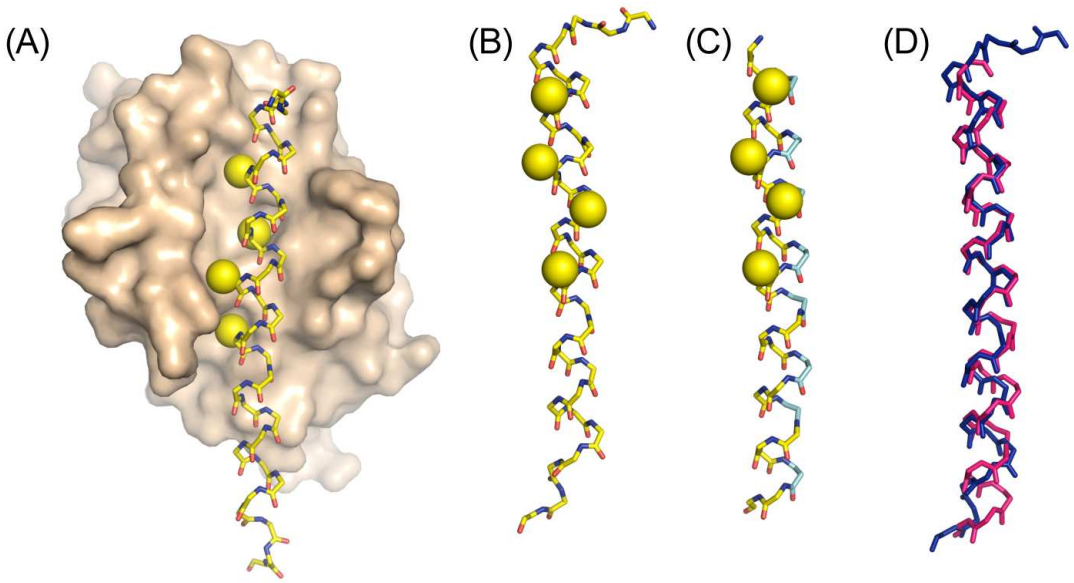

Figure S9. (A) Crystal structure of Bcl-x_L complexed with a peptide from the Bad BH3 domain (PDB: 1PQ1);[S4] Bcl-x_L is shown as a surface, and Bad BH3 as backbone sticks with spheres indicating the positions of the four conserved BH3 hydrophobic residues. (B) Another view of the Bad BH3 domain from (A). (C) Crystal structure of the helix formed by an α/β-peptide with an ααβαααβ backbone pattern (PDB: 2OXK);[S3] spheres indicate positions that would be occupied by the four conserved BH3 hydrophobic residues in oligomer **4**. (D) Overlay of the helical backbones from (B) in blue and (C) pink.

Materials and Methods

General. 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU), protected α-amino acids, and resins used in peptide synthesis were purchased from Novabiochem. Protected β^3-amino acids were purchased from PepTech except for Fmoc-β^3-hTrp(Boc)-OH which was prepared as previously described.[S5] 6-((4,4-difluoro-1,3-dimethyl-5-(4-methoxyphenyl)-4-bora-3a,4a-diaza-*s*-indacene-2-propionyl)amino)hexanoic acid, succinimidyl ester (BODIPY-TMR-X-SE) was purchased from Invitrogen. Proteinase K was purchased from Sigma. Pronase was purchased from Calbiochem. Solvents and all other reagents were purchased from Aldrich or Fisher and used as received. Reverse phase HPLC was carried out on Vydac analytical or preparative scale C_{18} columns using gradients between 0.1 % TFA in water and 0.1% TFA in acetonitrile.

Peptide Synthesis. Syntheses of α-peptide **1** and α/β-peptides **2**-**8** were carried out using standard Fmoc-solid phase peptide synthesis protocols by a combination of manual and automated methods. Briefly, Novasyn TGR resin (25 μmol) was suspended in CH_2Cl_2, allowed to swell for 1 hr, and then washed with DMF (3×). Fmoc-amino acid (75 μmol) and HBTU (28 mg, 74 μmol) were weighed into a separate vessel and dissolved in a 0.1 M solution of HOBT in NMP (0.75 mL). DIEA (26 μL, 150 μmol) was added, and the solution was allowed to react for 2 min and then added to the resin. The vessel was capped, placed on a bench top shaker, and agitated for 1-2 hr. The resin was washed with DMF (3×) and transferred to an Applied Biosystems Synergy 432A automated peptide synthesizer on which the remaining residues were coupled. The N-terminus of the resulting peptide was capped by treatment with 8:2:1 NMP / DIEA / Ac_2O. The resin was washed thoroughly (3×DMF, 3×CH_2Cl_2, 3×MeOH) and then dried under vacuum. Peptide was cleaved from resin by treatment with 94:2.5:2.5:1 TFA / H_2O / ethanedithiol / triisopropylsilane. The resin was filtered, washed with additional TFA, and the combined filtrates concentrated to ~2 mL under a stream of dry nitrogen. Crude peptide was precipitated from the cleavage mixture by addition of cold ether (45 mL). The mixture was centrifuged, decanted, and the remaining solid dried under a stream of nitrogen. Peptides were purified by preparative reverse phase HPLC on a C_{18} column. The identity and purity of the final products were confirmed by MALDI-TOF-MS and analytical HPLC, respectively. MALDI-MS (m/z): **1**: $[M+H]^+$ obsd = 3245.6 (calc. = 3245.6); **2**: $[M+H]^+$ obsd = 3343.2 (calc. = 3343.7); **3**: $[M+H]^+$ obsd = 3343.1 (calc. = 3343.7); **4**: $[M+H]^+$ obsd = 3343.9 (calc. = 3343.7); **5**: $[M+H]^+$ obsd = 3357.6 (calc. = 3357.7); **6**: $[M+H]^+$ obsd = 3357.8 (calc. = 3357.7); **7**: $[M+H]^+$ obsd = 3357.5 (calc. = 3357.7); **8**: $[M+H]^+$ obsd = 3343.7 (calc. = 3343.7). Peptide stock solutions were quantified by UV absorbance (ε_{280} = 6,970 M^{-1} cm^{-1}).[S6]

Synthesis of fluorescently labeled peptides. The Bak BH3 peptide (H-GQVGRQLAIIGDDINR-NH_2) was prepared as described above and labeled with BODIPY-TMR using previously reported methods.[S7] For preparation of BODIPY-TMR labeled **1**, **4** and **5**, analogues of each lacking the N-terminal acetyl group were prepared as described above and purified. A representative labeling reaction is described below. Peptide with free N-terminus (0.6 mg) was dissolved in 100 μL of 0.1 M $NaHCO_3$ at pH

8.3. BODIPY-TMR-X-SE (25 μL of a 10 mg/mL stock in DMSO) was added. The reaction was covered in foil and allowed to react for 8 hours with periodic vortexing. The reaction was then diluted into 3:1 water / acetonitrile with 0.1% TFA and purified by preparative reverse phase HPLC on a C_{18} column. The identity and purity of the final products were confirmed by MALDI-TOF-MS and analytical HPLC, respectively. MALDI-MS (m/z): **BODIPY-TMR-1**: $[M+H]^+$ obsd = 3695.9 (calc. = 3696.8); **BODIPY-TMR-4**: $[M+H]^+$ obsd = 3794.4 (calc. = 3794.9); **BODIPY-TMR-5**: $[M+H]^+$ obsd = 3808.7 (calc. = 3808.9). BODIPY-TMR labeled peptide stock concentrations were quantified by visible absorbance (ε_{535} = 50,000 M^{-1} cm^{-1} from Invitrogen product literature).

Protein expression and purification. Plasmids (pET vector) of human His-EK-Bcl-x_L (residues 1-196) and His-TEV-Mcl-1 (residues 171-327) were kind gifts from York Tomita (Georgetown). After transformation, BL21(DE3) *E. coli* cells were grown in Luria Broth with 15 μg/mL kanamycin to an OD_{600} of 0.6-0.9. Cells were induced with 0.4 mM isopropyl β-D-thiogalactoside (IPTG) for Mcl-1 or 1.0 mM IPTG for Bcl-x_L and incubated for 3-4 hr. After harvesting, cells were lysed in 50 mM Tris pH 8.0, 500 mM NaCl, 0.1% 2-mercaptoethanol (buffer 1) and centrifuged. The supernatant was applied to Ni-NTA agarose (Qiagen) and washed with buffer 1. The protein was eluted with 50 mM Tris pH 8.0, 200 mM NaCl, 200 mM imidazole. After reduction of salt concentration by dialysis, the protein was further purified with a FF Q column (GE healthcare) using a gradient between 25 mM Tris pH 8.0, 1 mM DTT and 25 mM Tris pH 8.0, 1 mM DTT, 1 M NaCl. The protein isolated from ion exchange chromatography was dialyzed into PBS containing 2 mM EDTA and 5 mM DTT. The sequences of the Bcl-x_L and Mcl-1 constructs used are given below.

Bcl-x_L

MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTDDDDKAMSMSQ
SNRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPEGTESEMETPSAIN
GNPSWHLADSPAVNGATGHSSSLDAREVIPMAAVKQALREAGDEFELRY
RRAFSDLTSQLHITPGTAYQSFEQVVNELFRDGVNWGRIVAFFSFGGAL
CVESVDKEMQVLVSRIAAWMATYLNDHLEPWIQENGGWDTFVELYG

Mcl-1

MGSHHHHHHHHGSDYDIPTTENLYFQGSEDELYRQSLEIISRYLREQAT
GAKDTKPMGRSGATSRKALETLRRVGDGVQRNHETAFQGMLRKLDIKNE
DDVKSLSRVMIHVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIEP
LAESITDVLVRTKRDWLVKQRGWDGFVEFFHVEDLEGG

Fluorescence polarization (FP) assays. Fluorescence polarization assays were conducted at room temperature in non-treated black polystyrene plates (Costar). All measurements were performed in duplicate wells. The assay buffer had the following composition: 20 mM phosphate, pH 7.4, 1 mM EDTA, 50 mM NaCl, 0.2 mM NaN_3, 0.5 mg/mL Pluronic F-68. Binding affinity of fluorescently labeled peptides was measured by titrating a fixed concentration of the labeled peptide with increasing concentrations of protein in 384-well plates with a final volume of 50 μL per well. The mixtures were

allowed to equilibrate for > 30 min and analyzed on an Envision 2100 plate reader. The data were fit using Graphpad Prism to a FP direct binding model.[S2] Competition fluorescence polarization assays were conducted using a previously described BODIPY-TMR-Bak BH3 peptide as a tracer (K_d = 2.5 nM for Bcl-x_L, K_d = 26 nM for Mcl-1).[S7] All competition experiments were conducted in 96-well plates with a total well volume of 120 μL per well by adding 2 μL of serial dilutions of inhibitor in DMSO to 118 μL of a solution in assay buffer containing final concentrations of 30 nM protein and 33 nM tracer for Bcl-x_L or 44 nM protein and 16 nM tracer for Mcl-1. Each assay plate also included 4 wells each of the following three controls: (1) 118 μL assay buffer + 2 μL DMSO; (2) 118 μL tracer (33 nM for Bcl-x_L, 16 nM for Mcl-1) + 2 μL DMSO; (3) 118 μL 30 nM protein and 33 nM tracer for Bcl-x_L or 44 nM protein and 16 nM tracer for Mcl-1 + 2 μL DMSO. The raw competition FP data were fit to sigmoidal dose response with variable slope using Graphpad Prism. The parameters describing the top, IC_{50}, and slope of each curve were allowed to float. Not all compounds showed complete inhibition at the highest peptide concentration, so the parameter for the bottom of the curves was set as a shared value for all compounds within a given dataset. The parameters obtained for the top and bottom of each curve were then used to normalize the data to % tracer bound. The normalized data were fit to exact analytical expressions for FP competitive binding[S1] with K_i as the only floating parameter for each compound. The lower bound for K_i measurable in the competition FP experiment (K_i = 1 nM for Bcl-x_L and K_i = 10 nM for Mcl-1) was determined by simulating competition curves using known K_d values for the tracer-protein complexes.[S1]

Protease susceptibility assays. Stock solutions of each protease were prepared in TBS (50 μg/mL proteinase K and 25 μg/mL pronase, based on weight to volume). Stock solutions of each peptide were prepared in TBS at 100 μM concentration as determined by UV absorbance. For each proteolysis reaction, 25 μL of peptide stock was mixed with 15 μL TBS. 10 μL of protease stock was added, the solution was mixed, and the reaction was allowed to proceed at room temperature. The reaction was quenched at the desired time point by addition of 100 μL of 1% TFA in water. 125 μL of the resulting quenched reaction was injected onto an analytical reverse phase HPLC, and the amount of starting peptide or α/β-peptide present quantified by integration of the peak at 220 nm. Duplicate reactions were run for each time point reported and half-lives determined by fitting time dependent peptide concentration to an exponential decay using GraphPad Prism. Crude samples for some time points were analyzed by MALDI-MS, and the products observed were used to identify amide bonds cleaved in the course of the reaction.

Circular dichroism. Circular dichroism measurements were carried out on an Aviv 202SF Circular Dichroism Spectrophotometer. Samples of each peptide were prepared at 25 μM concentration in 10 mM phosphate, pH 7. Spectra were recorded in a 2 mm cell with a step size of 1 nm and an averaging time of 5 sec. All spectra are background corrected against blank buffer measured in the same cell.

References for Supporting Information

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