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# Supplementary Materials: Engineering of Saposin C Protein Chimeras for Enhanced Cytotoxicity and Optimized Liposome Binding Capability

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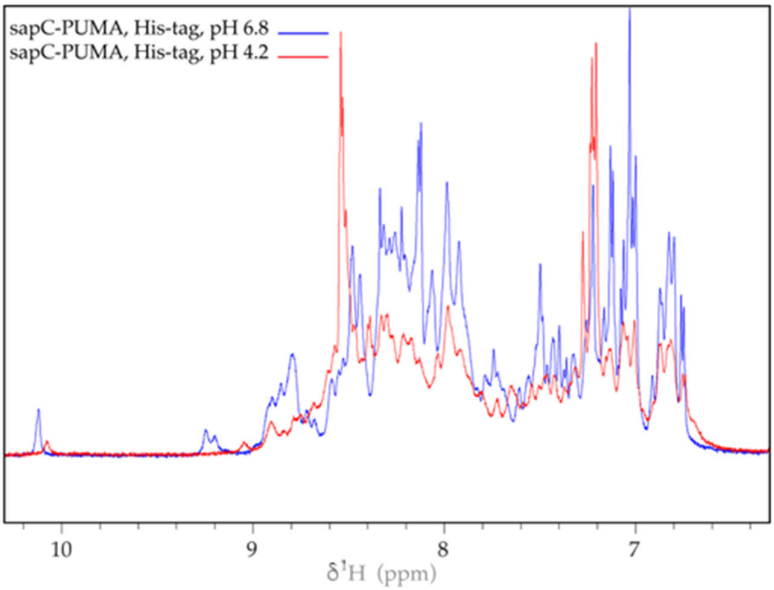


**Figure S1.** SapC-PUMA with His-tag aggregates upon acidification: SapC-PUMA with His-tag at pH 6.8 (blue, overall intensity from integration set to 100%) and pH 4.2 (red, intensity of 77% compared to the spectrum at pH 6.8).

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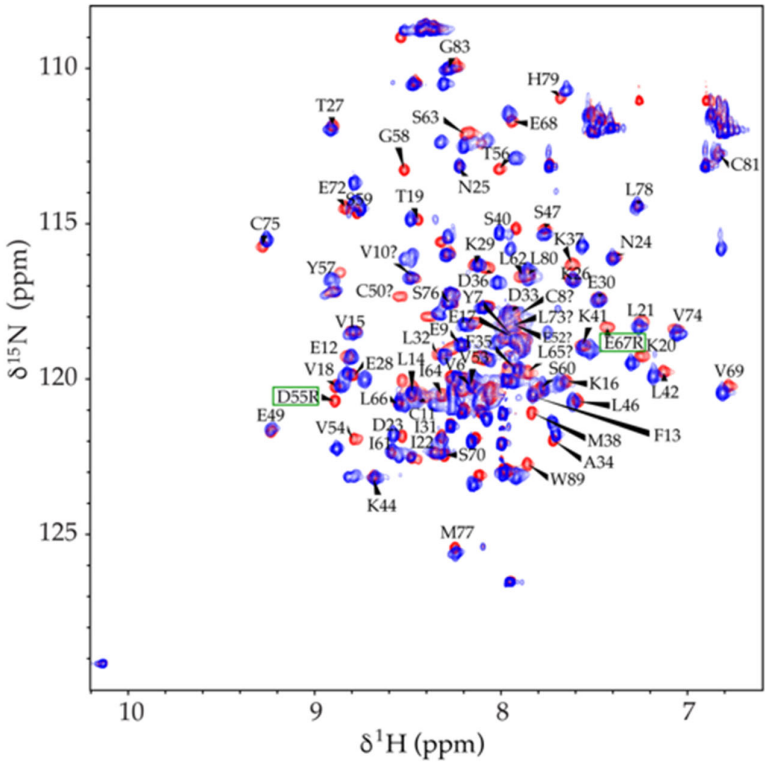


**Figure S2.** Double-mutant sapC-PUMA-DM shares structure identity to sapC-PUMA: [$^{1}$H,$^{15}$N]-sofast HMQC of sapC-PUMA (blue) and sapC-PUMA-DM (red) at pH 6.8 The amino acids that are mutated are shown with green rectangles. Other assignments are shown with the corresponding labels.

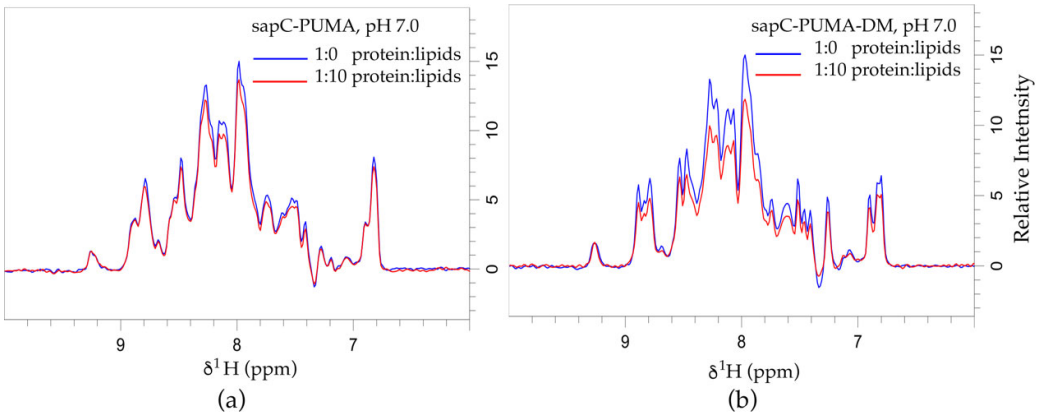


**Figure S3.** Increased binding of sapC-PUMA-DM to liposomes: 1D projections of [$^{1}$H,$^{15}$N]-sofast HMQC of sapC-PUMA (**a**) and sapC-PUMA-DM (**b**) in the absence (blue) and presence (red) of lipids at 1:10 protein:lipid molar ratio, pH 7. The binding of sapC-PUMA-DM is significantly larger compared to the wild-type chimera (8.5% vs. 28%) according to the decrease in signal intensity resulting from liposome binding.

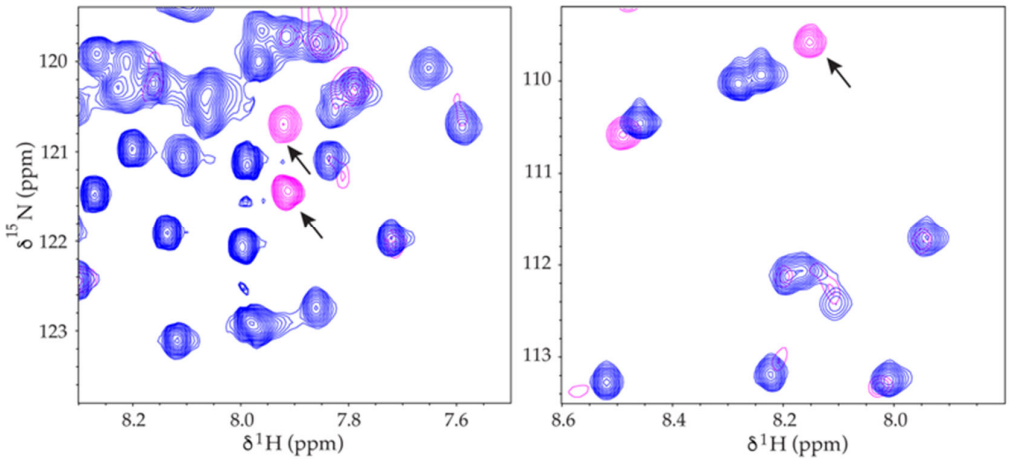


**Figure S4.** The PUMA region in sapC-PUMA-DM also binds Bcl-xL with high affinity: Selected regions of [$^{1}$H,$^{15}$N]-sofast HMQC spectra of sapC-PUMA-DM in the absence (blue) and in the presence (magenta) of 1:1 molar ratio of unlabeled Bcl-xL. The regions shown are equivalent to those appearing in Figure 8 c,d.

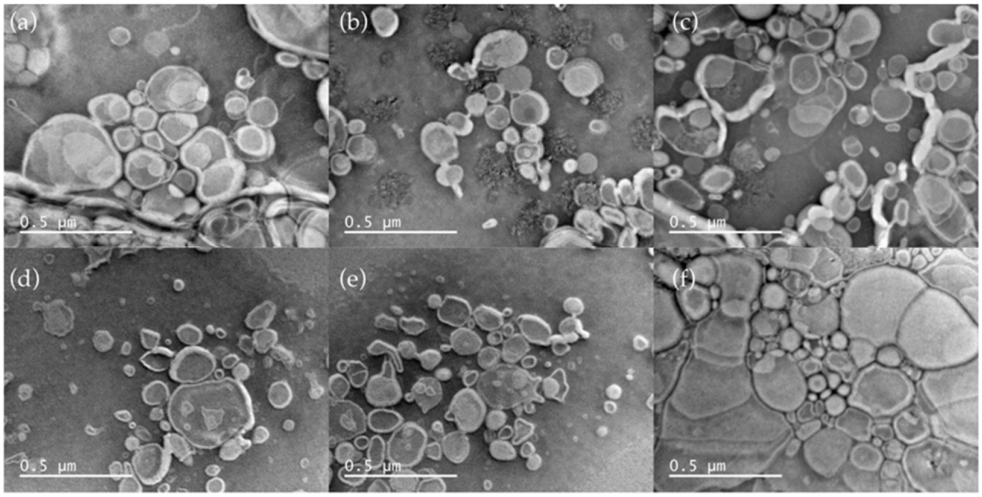


**Figure S5.** Liposome shape and size distortion in negative stained samples for TEM analysis: Micrographs (**a**)–(**c**) (same liposome sample) and (**f**) (different liposome sample but identical preparation procedure as (**a**)–(**c**)) were acquired after allowing the liposome solution to dry before staining. Micrographs (**d**,**e**) (same liposome samples as (**f**)) were allowed to dry for 20 min.