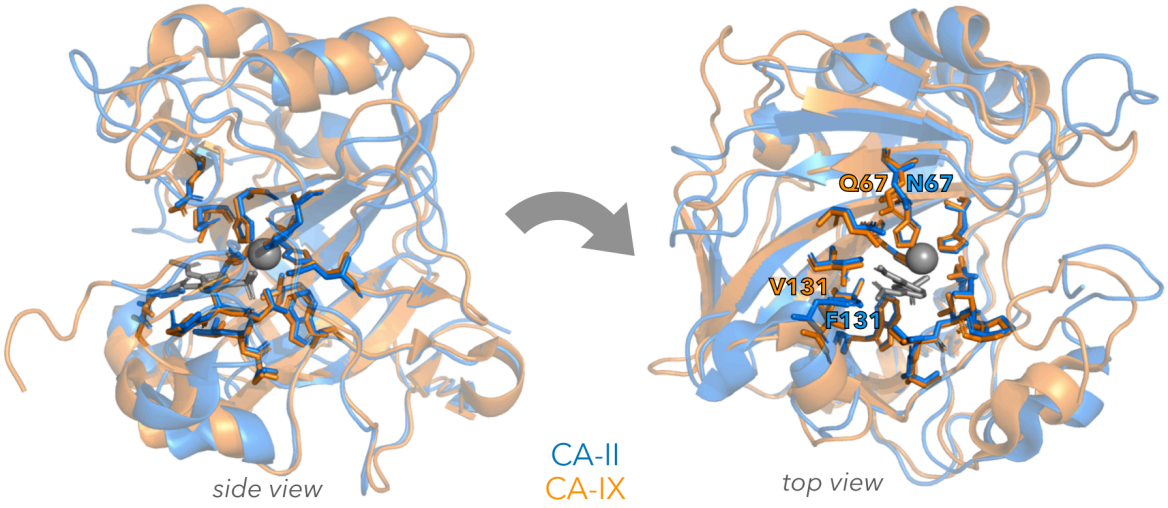

Figure S1. ***Carbonic anhydrase isoform homology. Related to STAR Methods.*** CA-II (blue) and CA-IX (orange) crystal structures with AAZ bound in the active site (PDB codes 3HS4 and 3IAI, respectively) were aligned. Side chains of amino acids within 5 Å of acetazolamide are shown and labeled.

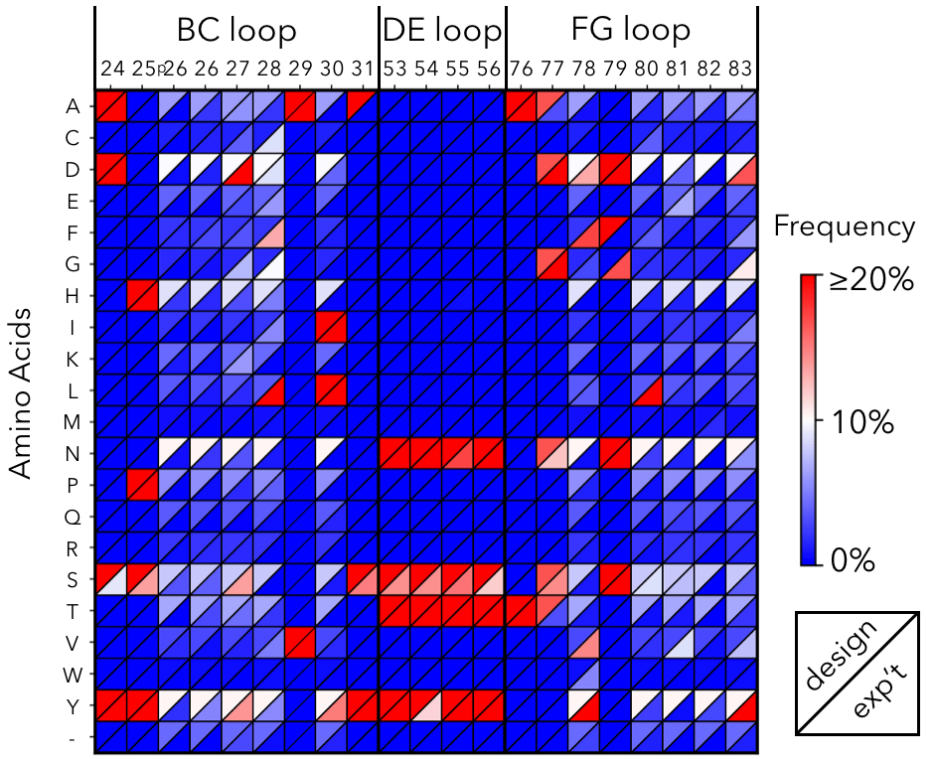

Figure S2. ***PriSM protein library matches design. Related to STAR Methods.*** Amino acid frequency at each site in the designed library (upper left triangle) and experimentally achieved library (lower right triangle). Data averaged across Cys28 and Cys80 libraries except sites 28 (only Cys80 diversity shown; 94% C in Cys28) and 80 (only Cys28 diversity shown; 89% C in Cys80). Median deviation is 0.1%.

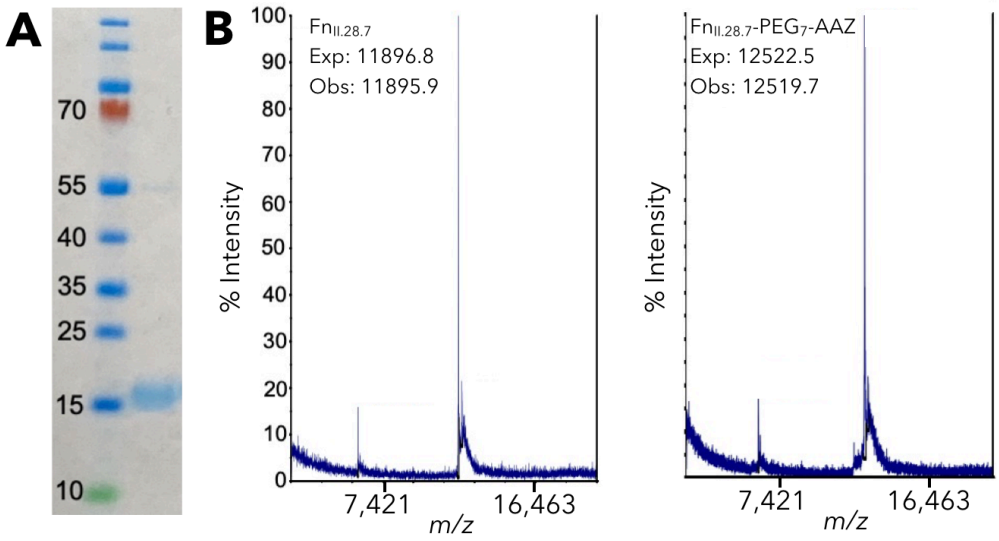

Figure S3. ***PriSMs are effectively produced via recombinant production and pharmacophore conjugation. Related to Figure 3.*** (A) The Fn component of the $Fn_{II\text{-}28.7}$-PEG_7-AAZ PriSM was recombinantly produced in *E. coli*, purified by metal affinity chromatography, and analyzed via electrophoresis. (B) Conjugation to maleimide-PEG_7-AAZ is validated via matrix-assisted laser desorption ionization mass spectrometry.

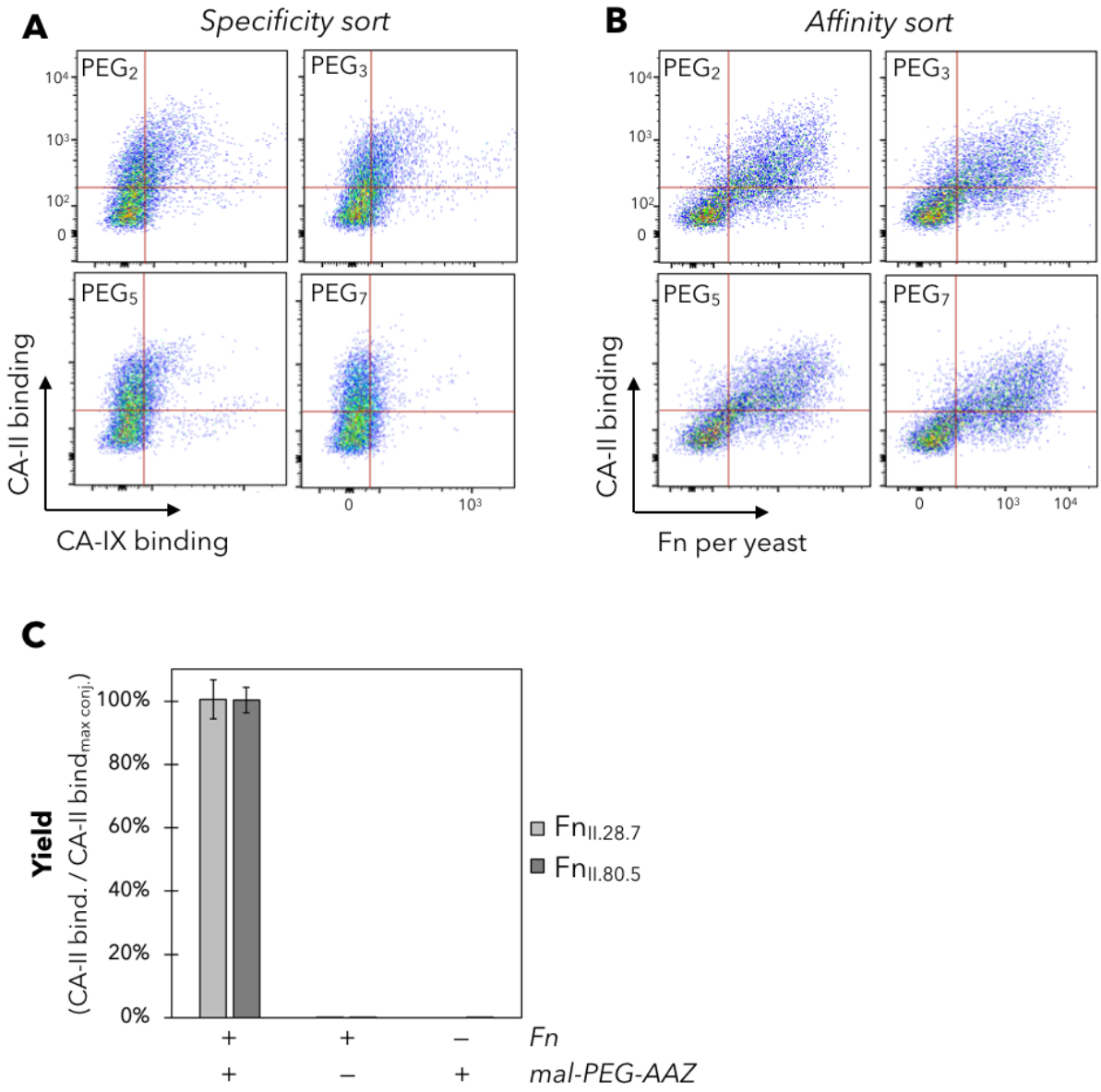

Figure S4. ***PEG-AAZ conjugation at Fn site 80 enables discovery of high affinity, isoform-specific PriSMs. Related to Figure 4.*** (A, B) The Cys80 Fn library was sorted for binding (A: specificity; B: affinity) to CA-II analogously to the Cys28 library in Figure 2. (C) Yeast displaying the indicated Fn clone (or negative control without Fn) were conjugated with 0 (-) or 8 (+) μM maleimide-PEG-AAZ, washed, and incubated with 10 nM CA-II. Binding was quantified by flow cytometry and compared to fully conjugated Fn treated with saturating levels of maleimide-PEG-AAZ. Data are presented as mean ± standard error of triplicate samples.

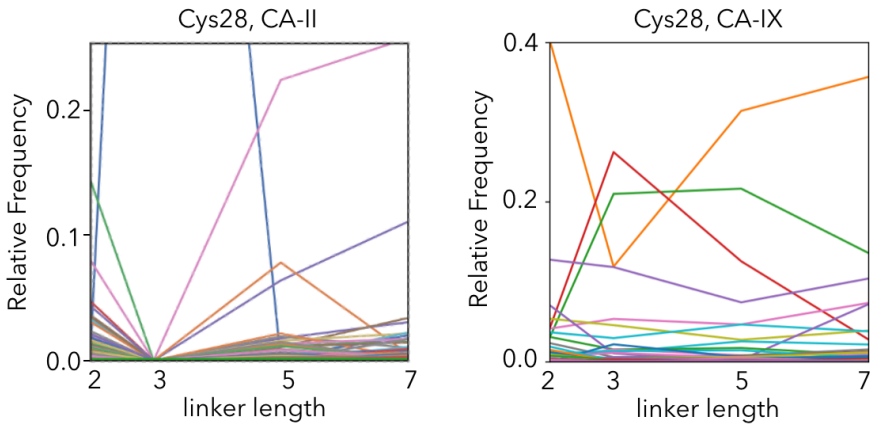

Figure S5. ***Clonal linker length preference. Related to Figure 5.*** Relative clonal frequency across different linker length campaigns for CA-II and CA-IX binders from Cys28 library.

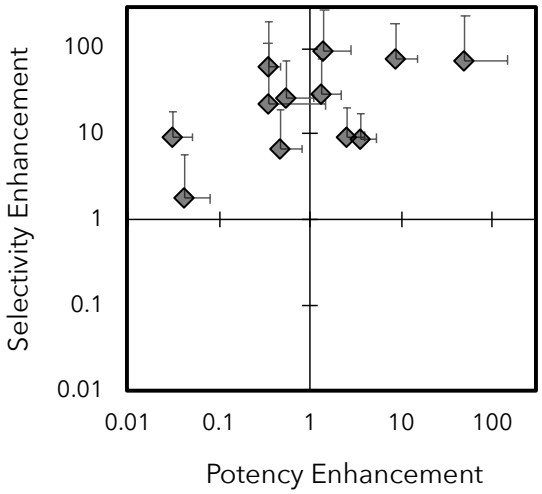

Figure S6. ***Conjugation to engineered Fn uniformly aids target selectivity and frequently aids target potency. Related to Figure 4.*** The selected Fn-PEG-AAZ variant from each campaign (CA-II or CA-IX; Cys28 or Cys80; PEG_2, PEG_3, PEG_5, or PEG_7; Figure 4) is compared to PEG_x-AAZ for inhibitory potency (K_i). *Selectivity enhancement* is the ratio of on-target:off-target potency between Fn-PEG-AAZ and PEG_x-AAZ. *Potency enhancement* is the ratio of on-target potency between Fn-PEG-AAZ and PEG_x-AAZ.

Table S1: ***DNA oligonucleotides for library construction and DNA amplification. Related to STAR Methods.***

Sequence	Description
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzxyzxyzxyzGYTxyzTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 10, 20*
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzxyzxyzxyzGYTMTCTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 10, IL
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzxyzxyzGYTxyzTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 9, 20*
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzxyzxyzGYTMTCTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 9, IL
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzxyzGYTxyzTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 8, 20*
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzxyzGYTMTCTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 8, IL
CGAGCCAGGAATTCACTGTTCCGGGAWMTWMTWMTWMTGCGACCATCAGCGGTCTGAAAC	DE loop, length 5
CGAGCCAGGAATTCACTGTTCCGGGAWMTWMTWMTGCGACCATCAGCGGTCTGAAAC	DE loop, length 4
CATTACCGTGTACGCTGTARCTRVTxyzRRCxyzxyzxyzxyzTCAAACCCAATCAGCATCAATTATCGCAC	FG loop, length 10
CATTACCGTGTACGCTGTARCTRVTxyzRRCxyzxyzxyzTCAAACCCAATCAGCATCAATTATCGCAC	FG loop, length 9
CATTACCGTGTACGCTGTARCTRVTxyzRRCxyzxyzTCAAACCCAATCAGCATCAATTATCGCAC	FG loop, length 8
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzxyzxyzTGCGYTxyzTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 10, C28 w 20*
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzxyzxyzTGCGYTMTCTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 10, C28 w IL
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzxyzTGCGYTxyzTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 9, C28 w 20*
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzxyzTGCGYTMTCTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 10, C28 w IL
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzTGCGYTxyzTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 8, C28 w 20*
ACTCTCTGACTATTTCTTGGGACKMTYMTxyzTGCGYTMTCTMTTACCGTATCACCTACGGCGAAAC	BC loop, length 8, C28 w IL
CATTACCGTGTACGCTGTARCTRVTxyzRRCTGCxyzxyzxyzTCAAACCCAATCAGCATCAATTATCGCAC	FG loop, length 10, C80
CATTACCGTGTACGCTGTARCTRVTxyzRRCTGCxyzxyzTCAAACCCAATCAGCATCAATTATCGCAC	FG loop, length 9, C80
CATTACCGTGTACGCTGTARCTRVTxyzRRCTGCxyzTCAAACCCAATCAGCATCAATTATCGCAC	FG loop, length 8, C80
CGACGATTGAAGGTAGATACCCATACGACGTTCCAGACTACGCTCTGCAG	Vector
ATCTCGAGCTATTACAAGTCCTCTTCAGAAATAAGCTTTTGTTCGGATCC	Vector
CGACGATTGAAGGTAGATACCCATACG	Full gene amplification primer
ATCTCGAGCTATTACAAGTCCTCTTC	Full gene amplification primer

* xyz = CDR diversity (x = 20% A, 19% C, 27% G, 34% T)(y = 44% A, 21% C, 12% G, 23% T)(z = 00% A, 00% C, 23% G, 77% T);

Table S2: ***Library design. Related to STAR Methods.*** The wild-type Fn sequence and library diversity is indicated at each diversified loop position. 20* refers to a biased composition that balances amino acid frequencies observed in human antibody complementary-determining regions, as well as evolved Fn domains. Loop lengths were varied by including or excluding codons at sites pre-26, 26, 55, 81, or 82 as indicated by -. Cysteine is conserved at either site 28 or site 80. At site 30, an equal mixture of two codons, 20* and IL, was used.

	BC Loop									DE Loop				FG Loop							
Name	24	25	p26	26	27	28	29	30	31	53	54	55	56	76	77	78	79	80	81	82	83
Wild-type	A	P	-	A	V	T	V	R	Y	S	K	S	T	T	G	R	G	D	S	P	A
Library (Cys28)	ADSY	HPSY	20*/-	20*/-	20*	C	AV	20*/IL	SY	NSTY	NSTY	NSTY/-	NSTY	AT	ADGNST	20*	DGNS	20*	20*/-	20*/-	20*
Library (Cys80)	ADSY	HPSY	20*/-	20*/-	20*	20*/IL	AV	20*/IL	SY	NSTY	NSTY	NSTY/-	NSTY	AT	ADGNST	20*	DGNS	C	20*/-	20*/-	20*

Table S3: ***Amino acid sequences for engineered Fn binders. Related to STAR Methods.***

Variant	Amino Acid Sequence
$Fn_{II.28.2}$	MASSSDSPRNLEVTNATPNSLTISWDDPKCAYYYRITYGETGGNSPSQEFTVPGNTNATISGLKPGQDYTITVYAVTGFSSLSNPISINYRT EIDKPSQGSHHHHHH
$Fn_{II.28.3}$	MASSSDSPRNLEVTNATPNSLTISWPWICPGMYHMPISINYRTEIDKPSQGSHHHHHH
$Fn_{II.28.5}$	MASSSDSPRNLEVTNATPNSLTISWDYPDCAIYYRITYGETGGNSPSQEFTVPGTNTNATISGLKPGQDYTITVYAVTDYSLSNSNPISINY RTEIDKPSQGSHHHHHH
$Fn_{II.28.7}$	MASSSDSPRNLEVTNATPNSLTISWDDSAACALYYRITYGETGGNSPSQEFTVPGTSYNATISGLKPGQDYTITVYAVADYSLQYSNPISIN YRTEIDKPSQGSHHHHHH
$Fn_{II.80.2}$	MASSSDSPRNLEVTNATPNSLTISWDDPAFAYYYRITYGETGGNSPSQEFTVPGYTNNATISGLKPGQDYTITVYAVTAEDCDSNPISINY RTEIDKPSQGSHHHHHH
$Fn_{II.80.3}$	MASSSDSPRNLEVTNATPNSLTISWDYSPYGAYYYRITYGETGGNSPSQEFTVPGYNSTATISGLKPGQDYTITVYAVTDFGCDSNPISINY RTEIDKPSQGSHHHHHH
$Fn_{II.28.5}$	MASSSDSPRNLEVTNATPNSLTISWDASGFAYYYRITYGETGGNSPSQEFTVPGYSSYATISGLKPGQDYTITVYAVTDFNCFSNPISINYR TEIDKPSQGSHHHHHH
$Fn_{II.80.7}$	MASSSDSPRNLEVTNATPNSLTISWDYHYLALYYRITYGETGGNSPSQEFTVPGNTYATISGLKPGQDYTITVYAVTDYDCISNPISINYRT EIDKPSQGSHHHHHH
$Fn_{IX.28.2}$	MASSSDSPRNLEVTNATPNSLTISWDYHQNGCAVSYRITYGETGGNSPSQEFTVPGYYDTYSATISGLKPGQDYTITVYAVTGYNDDSNP ISINYRTEIDKPSQGSHHHHHH
$Fn_{IX.28.3}$	MASSSDSPRNLEVTNATPNSLTISWDDHDKCVIYYRITYGETGGNSPSQEFTVPGYYDTYSATISGLKPGQDYTITVYAVANSDEDSNPISI NYRTEIDKPSQGSHHHHHH
$Fn_{IX.28.5}$	MASSSDSPRNLEVTNATPNSLTISWDDYQNCAVSYRITYGETGGNSPSQEFTVPGYYDTYSATISGLKPGQDYTITVYAVTGYNDDSNPI SINYRTEIDKPSQGSHHHHHH
$Fn_{IX.28.7}$	MASSSDSPRNLEVTNATPNSLTISWDDYLLCVFSYRITYGETGGNSPSQEFTVPGYTNTVTISGLKPGQDYTITVYAVTTYDLDSNPISINY RTEIDKPSQGSHHHHHH