SUPPLEMENTARY INFORMATION

METHODS

We examined the shape correlation parameter Sc [16] to see whether there was a correlation between toxin-neutralizing capacity, binding affinity (kD), and shape complementarity within the RTA-VHH interfaces described here. Although shape complementarity was high in all cases, it was not a strong predictor of RTA-neutralization or relative binding affinity. The more potent toxin-neutralizing F5 with an IC50 of 5 nM had a Kd of 2.2 X 10-9 and a Sc score of 0.828, while the weaker neutralizing VHH F8 with an IC50 of 300 nM had a similar Kd of 0.2 X 10-9 despite a similarly high Sc score of 0.802.

Supplementary Table 1

Data Collection		
Complex	RTA-F5	RTA-F8
APS Beamline	24-ID-E	24-ID-C
d_{min} (Å)	1.5	2.0
wavelength (Å)	0.979	0.979
No. of reflections	610453	880358
Average redundancy[a]	6.2 (6.2)	3.1(2.7)
$(I)/(\delta)$[a]	13.7(1.5)	11.4(0.95)
Completeness[a] (%)	99.7 (99.3)	96.8(98.7)
R_{merge}[a,b] (%)	8.4(56.5)	14.8(214.0)
CC*[c]	0.79	0.61
Refinement		
Bragg spacings (Å)	55.1-1.5	45.9-2.0
Space group	$C222_1$	$C222_1$
Cell parameters: *a, b,c* (Å)	78.4, 107.0, 111.9	88.5, 91.8, 95.1
R^d / R_{free}[e] (%)	17.8/19.5	20.4/25.7
No. of reflections	145133	24964
No. of waters	372	135
Rmsd bond length (Å)	0.019	0.006
Rmsd bond angle (°)	1.68	1.05
B-factors (Å^2): main chain/side chain	25.1 / 30.1	41.4 / 43.9
Ramachandran favored / allowed[f] (%)	97.2 / 100.0	97.9 / 100.0
PDB code	4Z9K	5E1H

[a] Values in outermost shell are given in parentheses.
[b] $R_{merge} = (\sum | I_i - \langle I_i \rangle |)/\sum | I_i |$, where I_i is the integrated intensity of a given reflection.
[c] CC*=Ö2CC1/2/1+CC1/2 , where CC1/2 is the correlation coefficient of two split data sets each derived by averaging half of the observations for a given reflection.
[d] $R = \sum | |F_o| - |F_c| | / \sum | |F_o| |$, where F_o and F_c denote observe and calculated structure factors, respectively.
[e] R_{free} was calculated using 5% of data excluded from refinement.
[f] Calculated using Molprobity

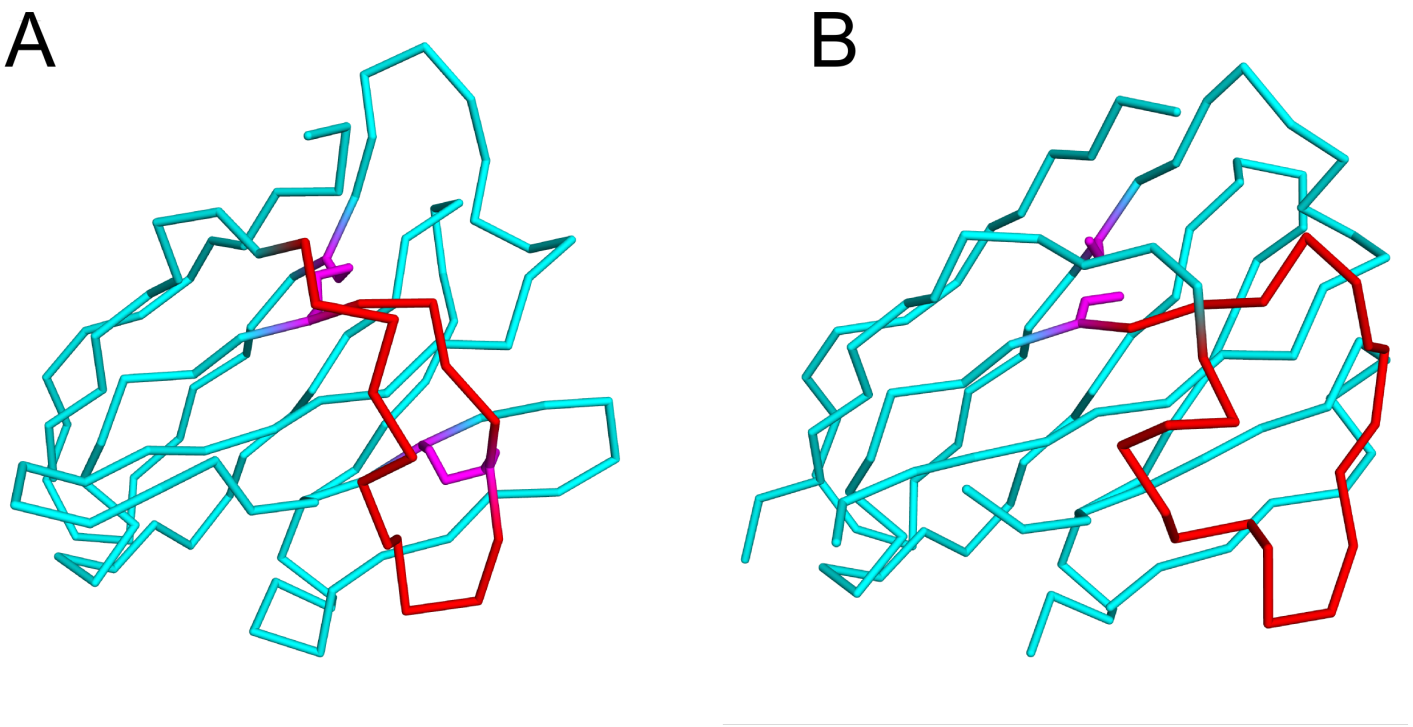
Figure Legends

Figure S1. Disulfide bond formation in VHH F5 and VHH F8. Depicted are the Cα-traces of (A) VHH F5 and (B) VHH F8. The CDR3 elements are colored red with the rest of the VHH colored in cyan. The disulfide bonds between residues Cys 22-Cys96 and Cys50-Cys97 in VHH F5 are shown in stick representation and colored magenta. Cysteines 22 and 96 not forming a disulfide bond in VHH F8 are also drawn in stick representation and colored cyan.

Figure S2. Amino acid sequence alignments of F8 with VHHs G12 and G11. (A) Sequence alignment of F8 with G12 and (B) F8 with G11. CDR1, 2, and 3 are highlighted with blue, yellow, and red, respectively. Sequence positions of residues involved in the hydrophobic interactions between the CDR3 and FR residues are highlighted with red asterisks above the sequence. Black asteriks below the sequence denote sequence identity. Figure made with ClustalW (jmb ref 51).

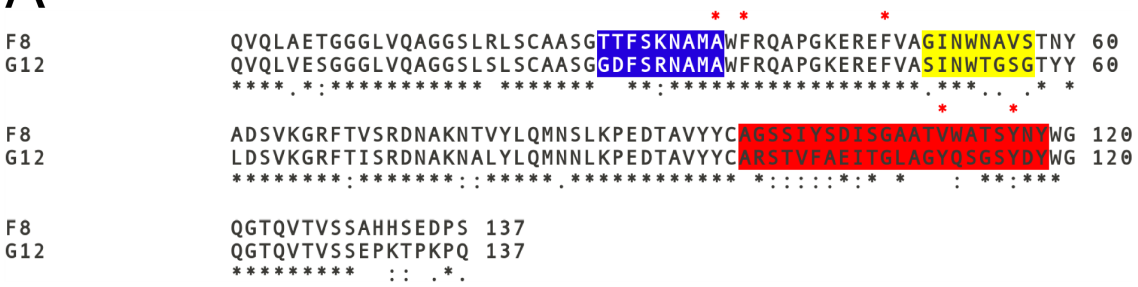
Figure S3. Conserved residues impacting CDR3 configuration. Shown are the super positioned Cα-traces of F8 with (A) four representative structural homologs possessing similarly constrained CDR3 configurations and F8 with (B) four representative structural homologs with a more extended CDR3. The CDR3 element for all homologs are colored dark gray, while the CDR3 elements of F8 is colored red. All VHHs are similarly oriented with the CDR3 in front. Sequence alignment of F8 with (C) the structural homologs in panel A (PDB: **2P42, 2X1Q, 4NBX, 4PPT)** and of F8 with (D) the structural homologs in panel B (PDB: **3EZJ, 4MQS, 4X7C, 4M3J)**. CDR 1, 2, and 3 are highlighted blue, yellow, and red, respectively. Residue positions potentially involved in the hydrophobic interactions between the CDR3 and FR residues are highlighted with red asterisks above the sequence. Black asteriks below the sequence denote sequence identity.

Supplementary Figure 1



Supplementary Figure 2

A



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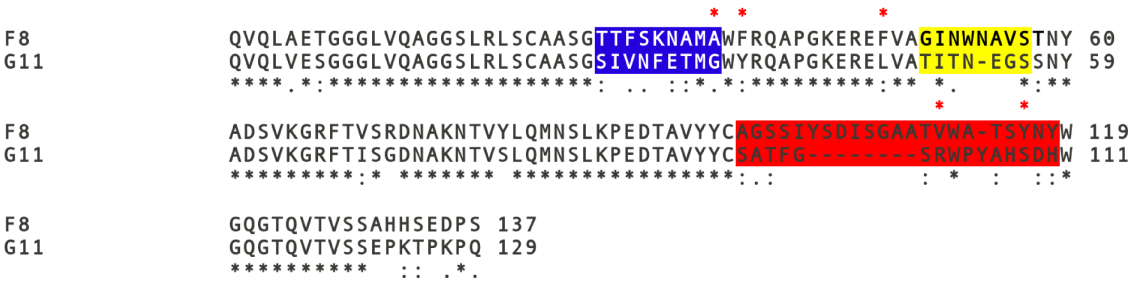
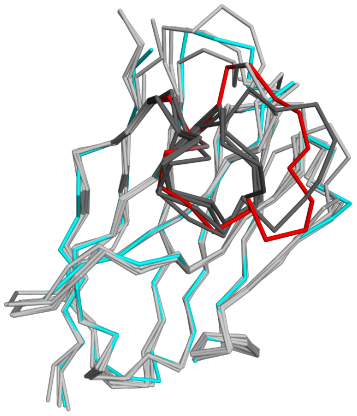


Supplementary Figure 3

A



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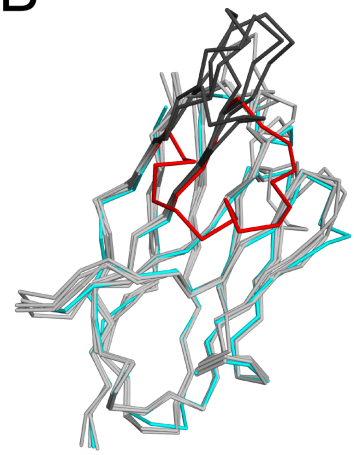

C

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F8          -------QVQLAETGGGLVQAGGSLRLSCAASGTTFSKNAMAWFRQAPGKEREFVAGINW     53
2P42        -----GSQVQLVESGGGLVQAGGSLRLSCAASGYAYTYIYMGWFRQAPGKEREGVAAMDS     55
2X1Q        -------QVQLQESGGGLVQPGGSLRLSCAASGRTFSNYRMGWFRQAPGKEREFVATISQ     53
4NBX        QPAMAQAQVQLVESGGGLAQAGGSLRLSCAASGRTFSMDPMAWFRQPPGKEREFVAAGSS     60
4PPT        -------QVQLVESGGGLVQAGGSLRLSCAASGYPHPYLHMGWFRQAPGKEREGVAAMDS     53
                  **** *:****.* ***********   .    *.**** ****** **    .

F8          NAVSTNYADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCAGSSIYSDISGAATVWA     113
2P42        GGGGTLYADSVKGRFTISRDKGKNTVYLQMDSLKPEDTATYYCAAGGYE--------LRD     107
2X1Q        SGAATAYADSVKGRFTFSRDNAKNLLYLEMLSLEPEDTAVYYCAASSRVFYT--EVLQTT     111
4NBX        TGRTTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAAPYG------ANWYR     114
4PPT        GGGGTLYADSVKGRFTISRDKGKNTVYLQMDSLKPEDTATYYCAAGGYE--------LRD     105
              . * **********.***:.** :**:* **:*****.****..

F8          TSYNYWGQGTQVTVSSAHHSEDPS--------137
2P42        RTYGQWGQGTQVTVSS----------------123
2X1Q        TGYDYWGQGTQVTVSS----------------127
4NBX        DEYAYWGQGTQVTVSSGQAGQGSEQKLISEED146
4PPT        RTYGHWGQGTQVTVSS----------------121
              *  ***********
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D

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F8          ----QVQLAETGGGLVQAGGSLRLSCAASGTTF---SKNAMAWFRQAPGKEREFVAGINW     53
3EZJ        ----QVQLQESGGGLVQAGGSLRLSCAASGSIF---SINSMDWDRQAPGKQRELVATITS     53
4MQS        GPGSQVQLQESGGGLVQAGDSLRLSCAASGFDFDNFDDYAIGWFRQAPGQEREGVSCIDP     60
4X7C        ----DVQLVESGGGLVQPGGSLRLSCAASGSIF---SIYAMGWYRQAPGKQRELVASISS     53
4M3J        ----QVQLQESGGGLVQPGGSLRLSCAASGSIS---SITTMGWYRQDPGKGRELVALINS     53
              :*** *:****** * *********      .  :: * ** **: ** *: *
                 *                                                  ***

F8          NAVSTNYADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCAGSSIYSDISGAATVWA     113
3EZJ        -GGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNANVK--------TWAG     104
4MQS        SDGSTIYADSAKGRFTISSDNAENTVYLQMNSLKPEDTAVYVCSAWTLF----------     109
4X7C        -GGGTNYADSVKGRFTISGDNAKNTVYLQMNSLKPEDTAVYYCKREDY--------SAYA     104
4M3J        -VGDTTYAGSVKGRFTISRDNAKNTVYLEMSSLKPEDTAVYYCNAFMS--------TNSG     104
              * ** *.*****:* ***:*****:*.********* *

F8          TSYNYWGQGTQVTVSSAHHSEDPS  137
3EZJ        MTRDYWGQGTQVTVS---------  119
4MQS        HSDEYWGQGTQVTVSS--------  125
4X7C        PPSGSRGRGTQVTVS---------  119
4M3J        RTGSFWGQGTQVTVS---------  119
              *:********
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