Supplementary Information

Table S1: Peptide map of RTA (Y80A V76M) used in HX-MS studies

Pep #	start	end	Pep #	start	end	Pep #	start	end	Pep #	start	end
1	1	12	**36**	93	104	**71**	163	169	**106**	218	233
2	13	21	**37**	93	108	**72**	166	169	**107**	218	241
3	13	25	**38**	94	100	**73**	166	172	**108**	219	226
4	22	25	**39**	94	108	**74**	169	172	**109**	219	233
5	26	33	**40**	103	108	**75**	170	174	**110**	221	233
6	26	38	**41**	104	108	**76**	173	182	**111**	222	233
7	29	38	**42**	105	108	**77**	176	182	**112**	227	233
8	34	60	**43**	105	110	**78**	179	182	**113**	227	241
9	38	60	**44**	109	118	**79**	183	187	**114**	228	241
10	39	46	**45**	109	119	**80**	183	188	**115**	233	241
11	39	56	**46**	109	123	**81**	183	189	**116**	233	244
12	39	58	**47**	119	123	**82**	183	191	**117**	233	249
13	39	60	**48**	120	127	**83**	183	205	**118**	234	244
14	57	60	**49**	124	127	**84**	188	205	**119**	234	245
15	59	62	**50**	124	130	**85**	189	205	**120**	234	247
16	59	69	**51**	124	134	**86**	190	205	**121**	234	249
17	61	69	**52**	124	136	**87**	190	207	**122**	241	244
18	61	70	**53**	128	134	**88**	192	205	**123**	242	245
19	63	69	**54**	128	136	**89**	192	208	**124**	242	247
20	70	73	**55**	131	136	**90**	196	205	**125**	242	249
21	70	74	**56**	131	152	**91**	206	211	**126**	244	249
22	70	75	**57**	134	145	**92**	206	215	**127**	245	249
23	71	75	**58**	135	147	**93**	206	217	**128**	246	249
24	73	80	**59**	135	152	**94**	206	218	**129**	248	254
25	73	92	**60**	137	147	**95**	208	215	**130**	248	255
26	74	80	**61**	137	148	**96**	208	217	**131**	248	256
27	74	92	**62**	137	152	**97**	208	218	**132**	250	254
28	76	80	**63**	147	152	**98**	209	215	**133**	250	255
29	76	92	**64**	148	151	**99**	209	217	**134**	250	256
30	81	92	**65**	148	152	**100**	209	218	**135**	256	268
31	81	93	**66**	149	152	**101**	212	217	**136**	257	268
32	85	92	**67**	153	162	**102**	212	218	**137**	258	268
33	93	100	**68**	153	165	**103**	213	217	**138**	259	268
34	93	102	**69**	154	165	**104**	218	221			
35	93	103	**70**	163	168	**105**	218	226			

Table S2. Data collection associated with X-ray crystal structures of V1C7 V_HHs

Complex	$V1C7_{G29R}$–RTA (high PO_4 condition)	$V1C7_{G29R}$–RTA (no PO_4 condition)
APS Beamline	24-ID-E	24-ID-E
d_{min} (Å)	2.5	2.5
wavelength (Å)	0.979	0.979
No. of reflections	617554	482323
Average redundancy[a]	10.3(9.6)	5.5(4.4)
$(I)/(\delta)$[a]	32.7(1.1)	38.3(2.2)
Completeness[a] (%)	100 (99.9)	99.9(99.7)
R_{merge}[a,b] (%)	11.0(279.3)	8.0(73.9)
CC*[c]	0.88	0.98
Refinement		
Bragg spacings (Å)	49.9-2.5	49.8-2.5
Space group	$P3_221$	$P3_221$
Cell parameters: *a,b,c* (Å)	64.9, 64.9, 215.9	64.9, 64.9, 215.4
R[d] / R_{free}[e] (%)	20.2/25.7	20.2/24.7
No. of reflections	19094	18990
No. of waters	24	47
Rmsd bond length (Å)	0.006	0.003
Rmsd bond angle (°)	0.98	0.75
B-factors (Å^2): main chain/side chain	91.1 / 93.4	74.3 / 77.5
Ramachandran favored / allowed[f] (%)	97.4 / 100.0	98.4 / 100.0
PDB code	5U4L	5U4M

Figure Legends

Figure S1. SPR analysis of V1C7 and V1C7$_{G29R}$. Sensorgrams from SPR analysis in which ricin-coated chips were probed with V1C7 or V1C7$_{G29R}$. Relative resonance units (RU) are compared across the treatments.

Figure S2. SPR analysis of V5C1 and V5C1$_{R29G}$. Sensorgrams from SPR analysis in which ricin-coated chips were probed with V5C1 or V5C1$_{R29G}$. Relative resonance units (RU) are compared across the treatments.

Figure S3. Homology models are similar to the initial V1C7–RTA template. Superposition of 10 randomly chosen V5C1–RTA homology models (thin lines) on the initial V1C7–RTA template (thick line).

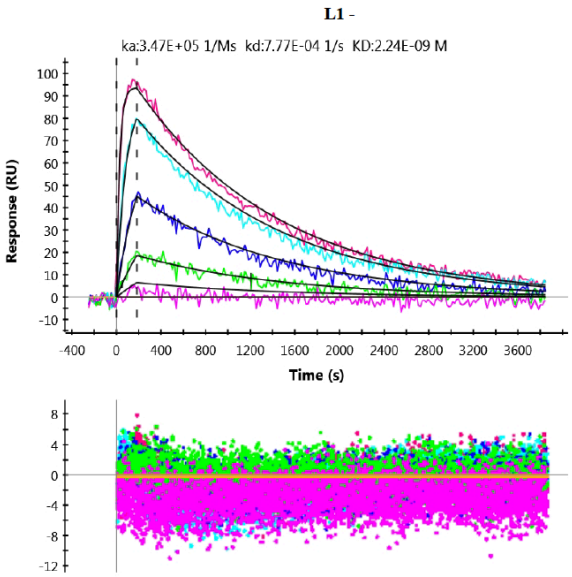
Figure S4. Differential protection, $\Delta(HX)^{-}$, as defined by equation 1, for RTA* induced by V_HH binding. (**Panel A**) V1C7, (**Panel B**) V1C7$_{G29R}$, (**Panel C**) V5C1, (**Panel D**) V5C1$_{R29G}$. The horizontal dashed lines at –0.1 denotes the threshold for strong protection. The dashed lines that follow the data denote the 99% confidence limit as described in the Materials and Methods. Gaps in the confidence limit indicate peptides with no available data. The horizontal axis is the ordinal number of each RTA* peptide arranged in order from the N-terminus to the C-terminus. The location of each peptide is listed in **Supplemental Table S1**.

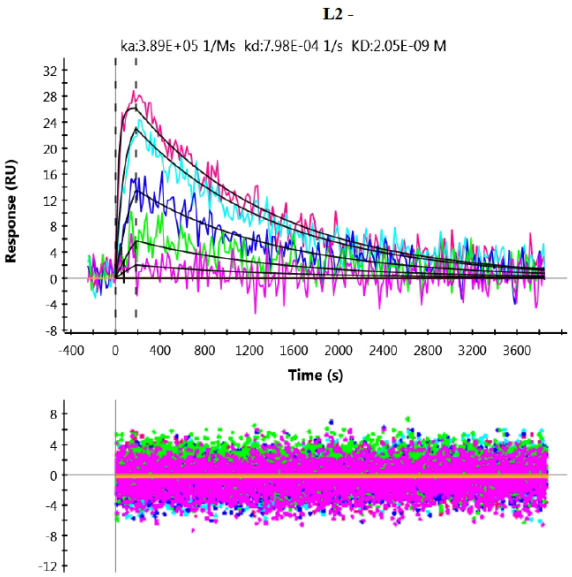
Fig S5. Close-up of the interactions between V1C7$_{G29R}$ and RTA. (**A**) High phosphate crystal form of RTA-V1C7$_{G29R}$. RTA (slate), V1C7$_{G29R}$ (gray), and symmetry-related RTA (light blue) are drawn as ribbon diagrams. The bound phosphate molecule is drawn as sticks with oxygen atoms colored red and the phosphorous atom colored orange. (**B**) Interaction of symmetry-related RTA residues Arg 196 and Arg 235 (light blue) with RTA's Glu 67 (violet) from the high-phosphate crystal form. (**C**) Superposition of the no-phosphate crystal form of RTA-V1C7$_{G29R}$ (RTA colored magenta and V1C7$_{G29R}$ colored cyan) with the high-phosphate crystal form of RTA-V1C7$_{G29R}$ (RTA colored violet and V1C7$_{G29R}$ colored gray). (**D**) Interaction of symmetry-related RTA residues Arg 196 and Arg 235 (light blue) with RTA's Glu 67 (magenta) from the no-phosphate crystal form. All side chains are drawn as sticks and color coordinated to their respective main chain. Key distances between atoms are displayed as red dashes.

Figure S6. Possible mechanism by which V5C1, as opposed to V1C7, achieves greater neutralization of ricin toxin. (Panel A) In the V1C7–RTA crystal structure (PDB: 5J56), CDR3 residues Ser111$_{V1C7}$ and Gln113$_{V1C7}$ interact favorably with Gln160$_{RTA}$, for a total interaction energy of -0.7 REUs. This interaction may lock CDR3 away from RTA's epitope located between α-helices D and E. (**Panel B**) In V5C1's representative model, Tyr111$_{V5C1}$ and Thr113$_{V5C1}$ have no interaction with Gln160$_{RTA}$ (0.0 REU). This suggests that V5C1's CDR3 adopts an alternative conformation that contacts the epitope and leads to ricin neutralization, but this conformation departs too much from the V1C7 template to be sampled by our modeling.

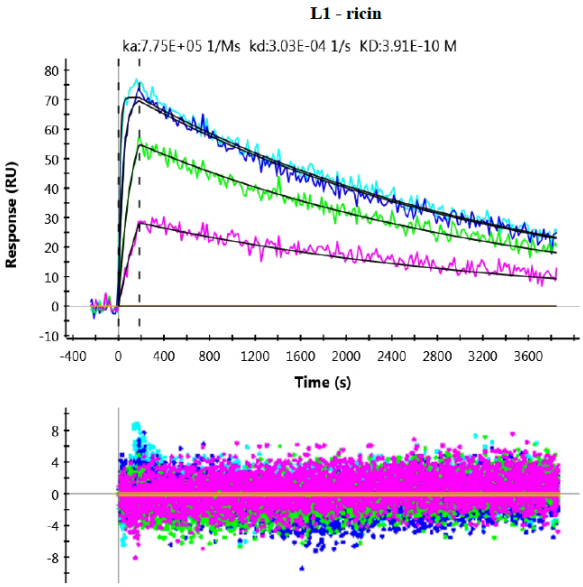
Figure S1.

A. V1C7





B. V1C7 (G29R)



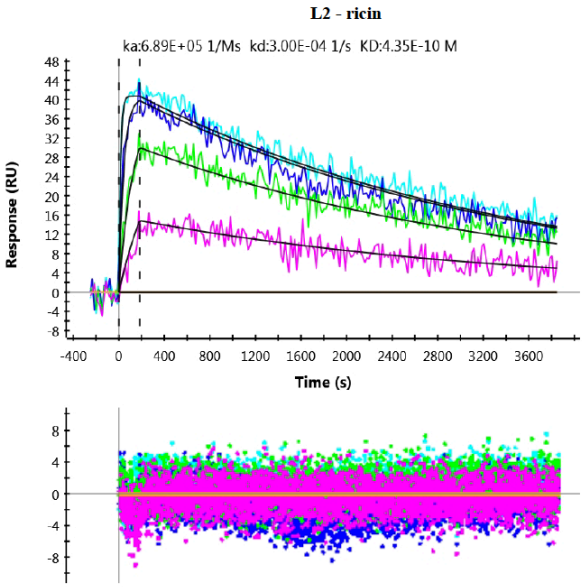
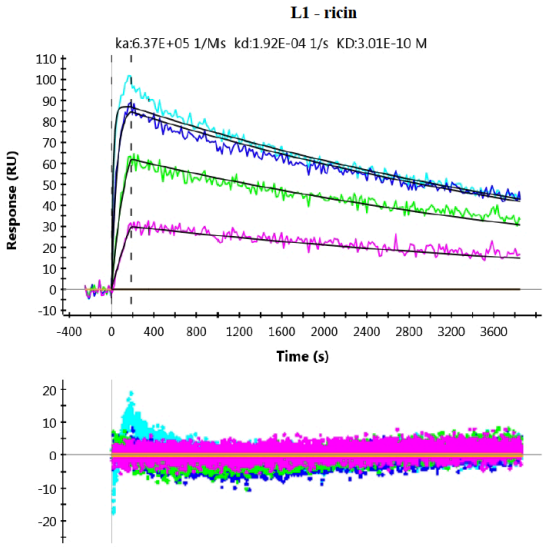
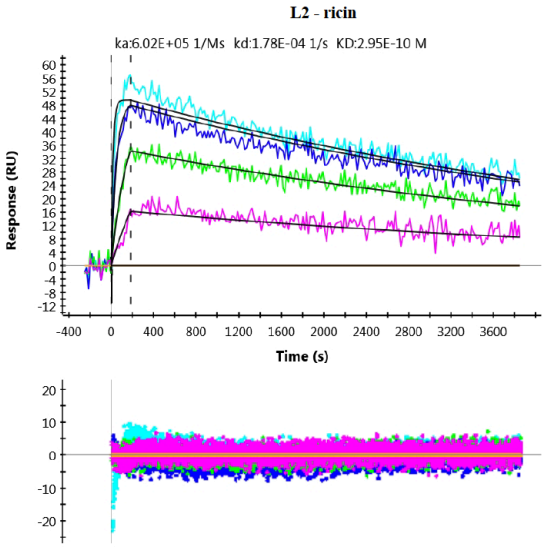


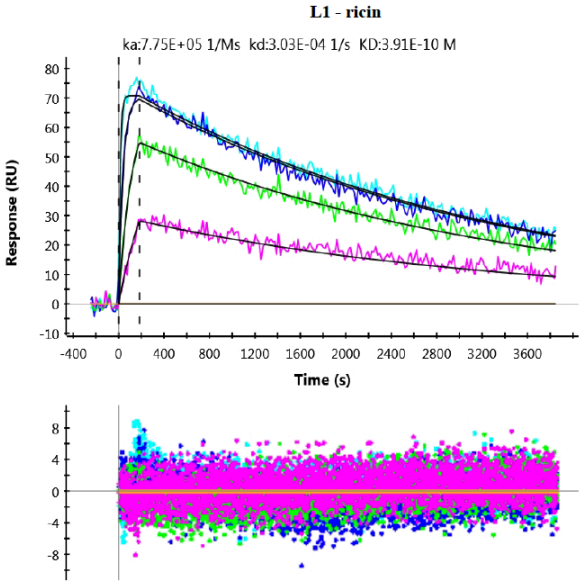
Figure S2.

A. V5C1





B. V5C1 R29G



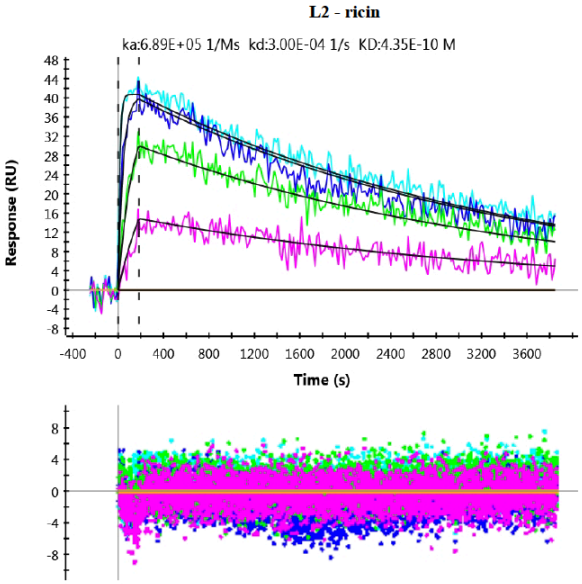


Figure S3

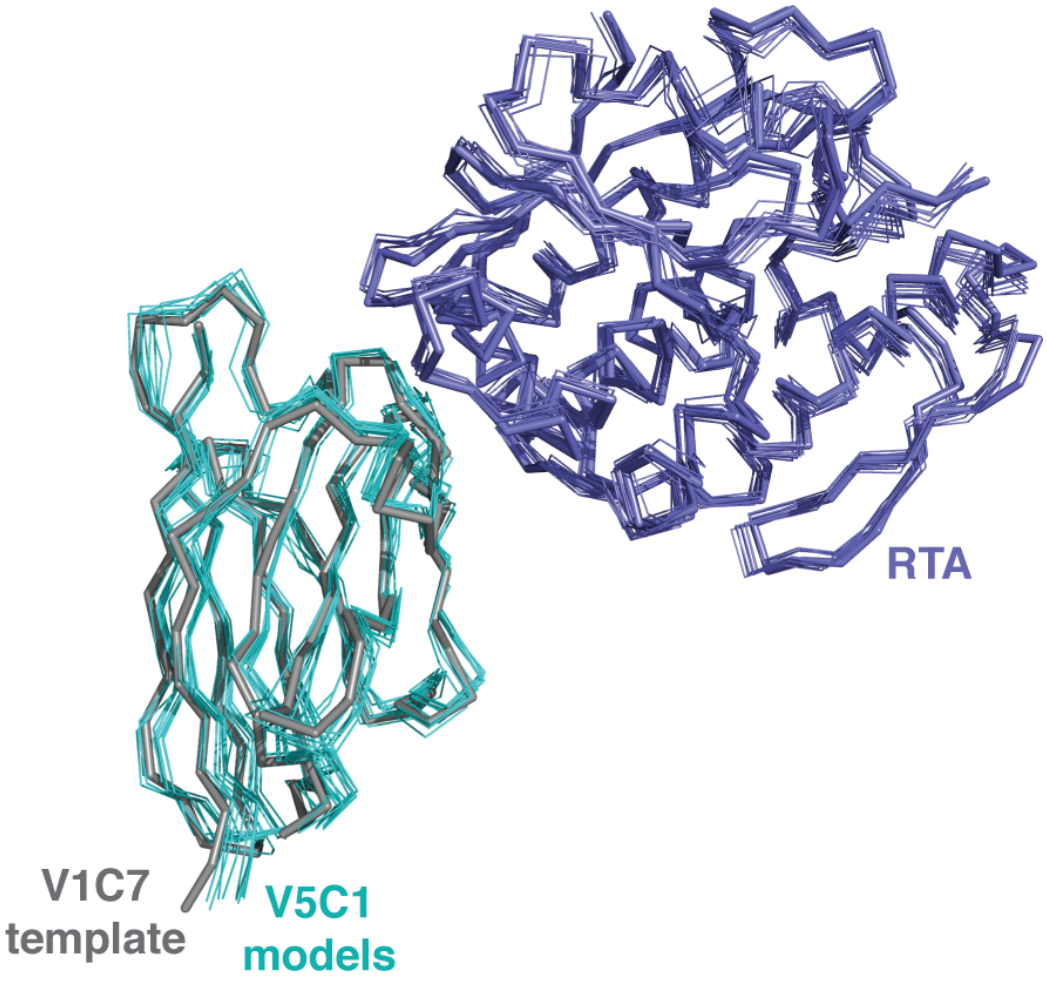


Figure S4

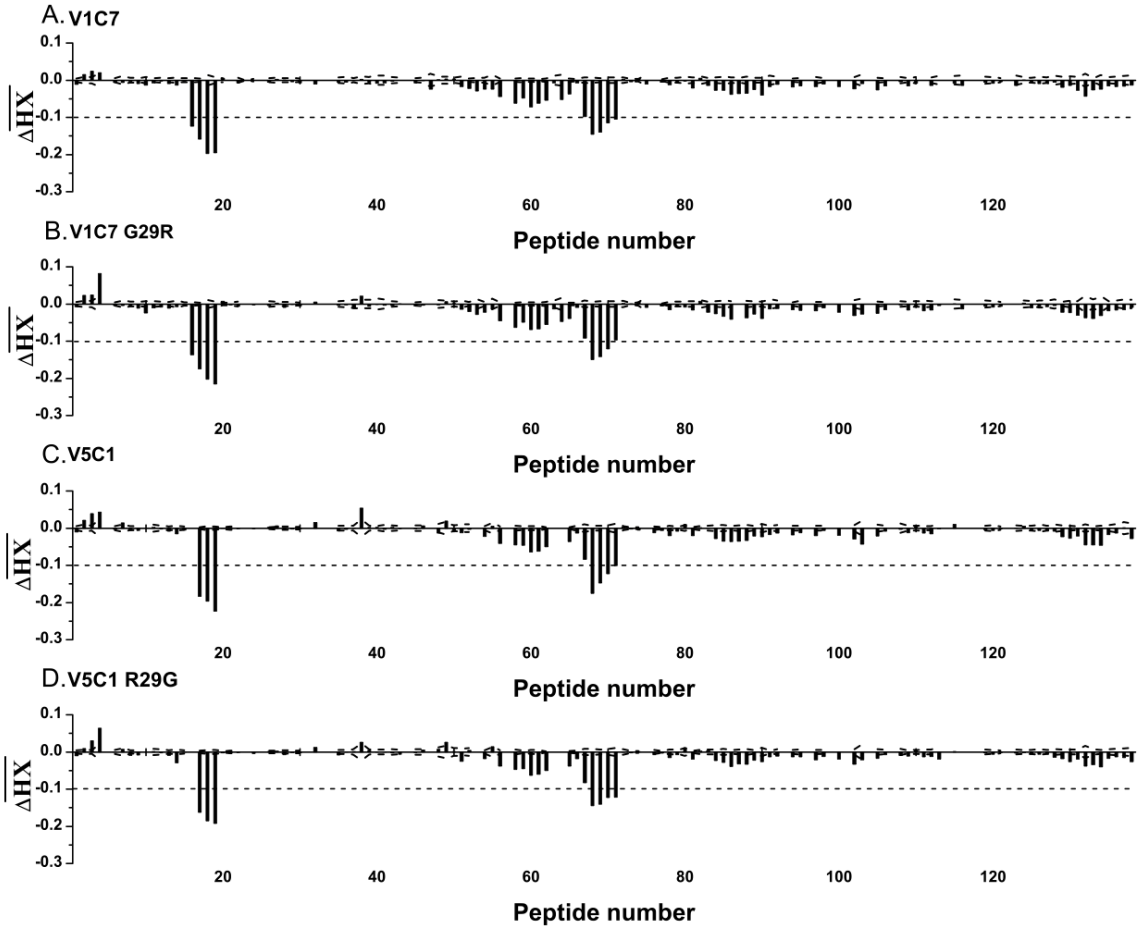

A. V1C7
B. V1C7 G29R
C. V5C1
D. V5C1 R29G
ΔHX
0.1
0.0
-0.1
-0.2
-0.3
20
40
60
80
100
120
Peptide number

Figure S5.

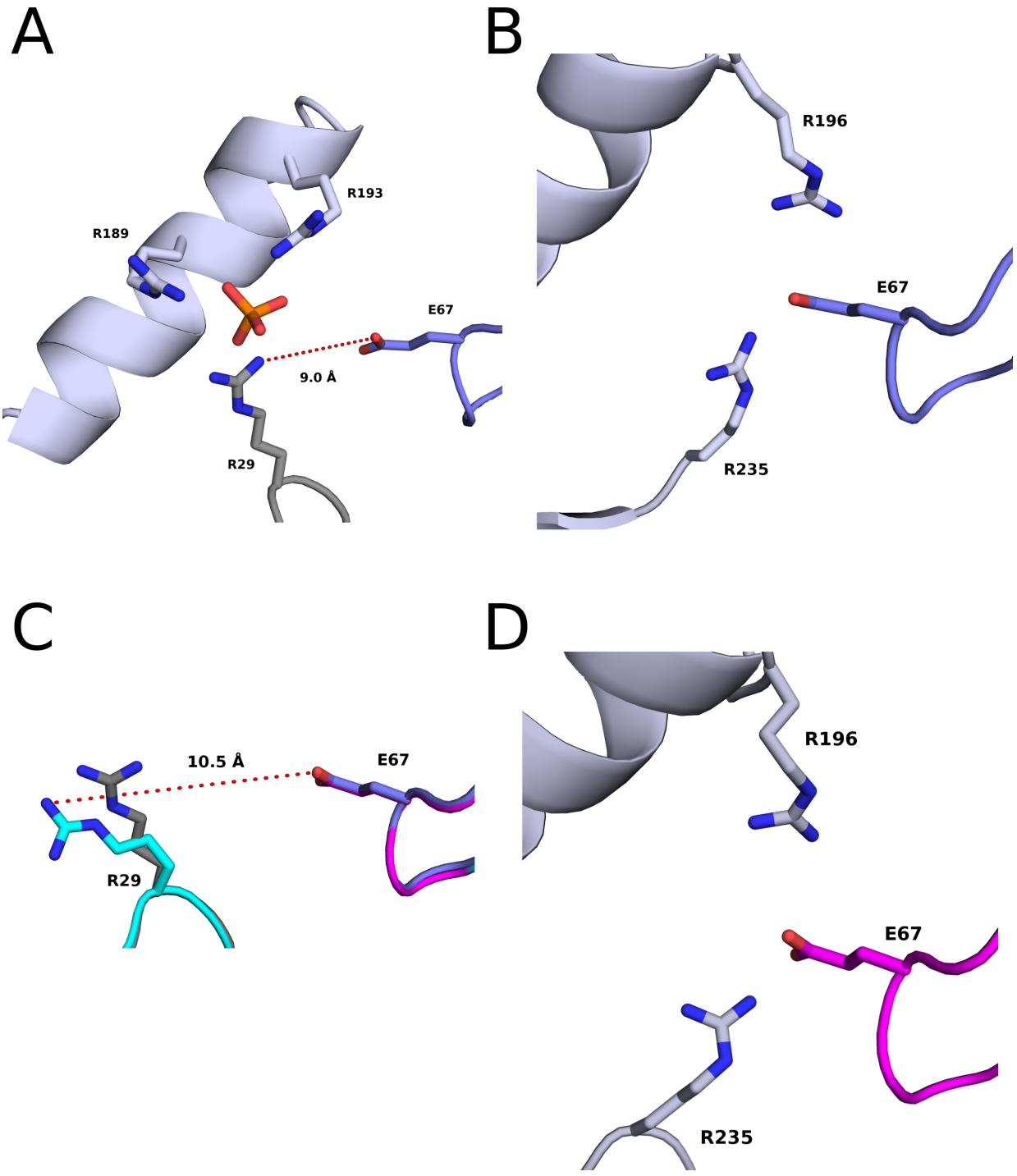

A
R193
R189
E67
9.0 Å
R29
B
R196
E67
R235
C
10.5 Å
E67
R29
D
R196
E67
R235

Figure S6.