Supplementary Table 1. SAXS data collection and analysis of TcdB holotoxin

	pH = 7.4	**pH = 5.0**
Data collection parameters		
Instrument	SSRL BL4-2	SSRL BL4-2
Type of Experiment	SEC-SAXS	SEC-SAXS
Defining slits size (H mm × V mm)	0.25× 0.20	0.25× 0.20
Detector distance (m)	2.5	2.5
Detector	Pilatus3 X 1M	Pilatus3 X 1M
Beam energy (keV)	12.4	12.4
q range ($Å^{-1}$)	0.0056–0.40	0.0046–0.40
Exposure time/frame (s)	1	1
Frames per data set	500	500
Temperature (K)	298	298
SEC parameters		
SEC column	Superdex 200 Increase PC 3.2/300	Superdex 200 Increase PC 3.2/300
Sample concentration (mg/ml)	20	20
Injection volume (µL)	15	30
HPLC flow rate (mL/min)	0.05	0.05
Buffer	PBS, pH 7.4, and 5 mM DTT	20 mM sodium acetate, 50 mM NaCl, pH 5.0, and 5 mM DTT
Software employed		
Primary data reduction	SasTool	SasTool
Data processing	PRIMUS	PRIMUS
Structural parameters		
Guinier analysis		
$I(0)$	402.45 ± 1.50	58.06 ± 1.07
R_g (Å)	59.20 ± 3.22	61.30 ± 1.41
q_{min} ($Å^{-1}$)	0.0135	0.0131
$qR_{g\,max}$	1.3	1.29
Coefficient of correlation, R^2	0.9995	0.9998
P(r) analysis		
$I(0)$	416.40	59.36
R_g (Å)	63.18	64.73
D_{max} (Å)	205.0	233.0
q range ($Å^{-1}$)	0.0135-0.257	0.0106-0.257
χ^2 (total estimate from GNOM)	0.239 (0.718)	0.224 (0.770)
Porod volume estimate ($Å^3$) (ratio Vp/calculated M)	474000 (1.75)	529000 (1.96)

V, M using the Fischer method (ratio of *M* to expected)	307063, 253338 (0.94)	322532, 266099 (0.99)
Curve fitting using atomic model		
Software employed	*CRYSOL*	*CRYSOL*
q range for all modeling	0.0171-0.200	0.0171-0.200
(No constant subtraction)		
χ^2, *P*-value	12.642, 0.00	0.293, 0.00
Predicted R_g (Å)	63.68	64.27
Vol (Å), Ra (Å), Dro (e/Å^3)	328548, 1.400, 0.000	305434, 1.400, 0.030
(Constant subtraction allowed)		
χ^2, *P*-value	11.461, 0.00	0.293, 0.00
Predicted R_g (Å)	63.76	64.27
Vol (Å), Ra (Å), Dro (e/Å^3)	305434, 1.800, 0.000	308736, 1.400, 0.030

Supplementary Table 2. Relative quantum yield and steady state anisotropy for dye-labeled VHHs.

Standard Error of Measurement

Alexa-555	Relative Quantum Yield		Anisotropy	
	pH 7	pH 5	pH 7	pH 5
Free Dye	1.00 ± 0.02	0.93 ± 0.02	0.209 ± 0.002	0.211 ± 0.008
VHH B39	1.79 ± 0.06	1.82 ± 0.05	0.244 ± 0.003	0.255 ± 0.003
VHH–TcdB	1.78 ± 0.06	1.79 ± 0.06	0.236 ± 0.006	0.272 ± 0.002

Alexa-647	Relative Quantum Yield		Anisotropy	
	pH 7	pH 5	pH 7	pH 5
Free Dye	1.00 ± 0.03	0.99 ± 0.02	0.155 ± 0.003	0.141 ± 0.010
VHH F7	1.56 ± 0.01	1.55 ± 0.04	0.260 ± 0.008	0.245 ± 0.010
VHH–TcdB	1.62 ± 0.03	1.56 ± 0.01	0.245 ± 0.010	0.305 ± 0.008

Supplementary Table 3. Protein-protein interactions in the $TcdB^{1072–1433}$–5D, GTD–E3, and GTD ^{VPI10463}–7F complexes.

Interactions between $TcdB^{1072–1433}$ and 5D

5D residues	$TcdB^{1072–1433}$ residues	Type of interaction
T28	N1110	Hydrogen bond (sc-sc)
Y50	E1307	Hydrogen bond (sc-sc)
S52	E1311	Hydrogen bond (sc-sc)
A53	E1311	Hydrogen bond (mc-sc)
S54	E1311	Hydrogen bond (sc-sc)
A55	E1311	Hydrogen bond (mc-sc)
R56	E1311	Hydrogen bond (sc-mc)
T57	E1311 N1332	Hydrogen bond (sc-sc) Hydrogen bond (sc-sc)
Y31 Y32 F101	P1105 L1107 L1112 T1306 Y1308	vdW (sc-sc)
S104 S105 V106	T1305 Q1330 Y1331 V1356 T1357 I1358	vdW (sc-sc)
R108	R1310 N1332	Hydrogen bond (sc-sc) Hydrogen bond (sc-sc)

Interactions between GTD and E3

E3 residues	GTD residues	Type of interaction
W47	D29	Hydrogen bond (mc-sc)
N56	D51 S54	Hydrogen bond (sc-water-sc) Hydrogen bond (sc-water-sc)
S60	E33	Hydrogen bond (sc-sc)
D61	K48	Salt bridge (sc-sc)
K64	D51	Hydrogen bond (sc-water-sc)
K96	E23 Y63	Hydrogen bond (sc-sc) Hydrogen bond (sc-sc)
R101	D22	Salt bridge (sc-sc)
T35 L45 W47 S50 I58	V25 A26 A30 L55	vdW (sc-sc)

Interactions between $GTD^{VPI10463}$ and 7F

7F residues	GTD residues	Type of interaction
S27	K216	Hydrogen bond (sc-water-sc)
S30	Y214	Hydrogen bond (sc-mc)
T31	K216 N151	Hydrogen bond (sc-water-sc) Hydrogen bond (mc-sc)
T33	Y538	Hydrogen bond (sc-sc)
T37	R158	Hydrogen bond (sc-sc)
W47	R158	Hydrogen bond (sc-sc)
S50	E155	Hydrogen bond (sc-sc)
S53	S148 D152	Hydrogen bond (sc-sc) Hydrogen bond (mc-sc)
G54	D152	Hydrogen bond (mc-sc)
K58	R158	Hydrogen bond (sc-water-mc)
N73	E213	Hydrogen bond (sc-water-sc)
N99	E147 N151	Hydrogen bond (sc-sc) Hydrogen bond (sc-sc)
A100	N537	Hydrogen bond (mc-sc)
S102	E147	Hydrogen bond (sc-water-sc)
S104	N537 N537 Y538	Hydrogen bond (mc-water-mc) Hydrogen bond (sc-mc) Hydrogen bond (sc-mc)
A100 V101	I150 L154 Y534 Y538	vdW (sc-sc)

"vdW" stands for van der Waals interaction. "mc" and "sc" indicates whether the contact was mediated by main-chain or side-chain atoms.