Table S1. Cryo-EM data collection, image processing, and validation statistics, Related to STAR Methods

	miniV1_apo (EMD-23136) (PDB-7L2P)	miniV1_DkTx pre-bound (EMD-23139) (PDB-7L2S)	miniV1_DkTx singly-bound (EMD-23161) no model	miniV1_DkTx pre-open (EMD-23138) (PDB-7L2R)	miniV1_DkTx partial open (EMD-23140) (PDB-7L2T)	miniV1_DkTx open (EMD-23141) (PDB-7L2U)	miniV1_RTX-NMDG a (EMD-23143) (PDB-7L2W)	miniV1_RTX-NMDG b (EMD-23142) (PDB-7L2V)	miniV1_RTX-NMDG c (EMD-23144) (PDB-7L2X)	FLV1_pH6 a (EMD-23129) (PDB-7L2I)	FLV1_pH6 b (EMD-23131) (PDB-7L2K)	FLV1_pH6 c (EMD-23130) (PDB-7L2J)	FLV1_pH5.5_RTX (EMD-23135) (PDB-7L2O)
Data Collection/Processing													
Microscope	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Talos Arctica	Talos Arctica	Talos Arctica	Talos Arctica
Detector	Gatan K2	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K2	Gatan K2	Gatan K2	Gatan K2
Voltage (kV)	300	300	300	300	300	300	300	300	300	200	200	200	200
Magnification	22,500	105000	105000	105000	105000	105000	105000	105000	105000	36,000	36,000	36,000	36,000
Defocus Range (μm)	-0.2 to -2.0	-0.5 to -3.0	-0.5 to -3.0	-0.5 to -3.0	-0.5 to -3.0	-0.5 to -3.0	-0.5 to -3.0	-0.5 to -3.0	-0.5 to -3.0	-0.5 to -2.5	-0.5 to -2.5	-0.5 to -2.5	-0.5 to -2.8
Pixel Size (Å)	1.059	0.8488	0.8488	0.8488	0.8488	0.8488	0.835	0.835	0.835	1.15	1.15	1.15	1.15
Total Electron Dose ($e^-/Å^2$)	72	67	67	67	67	67	68.4	68.4	68.4	76.8	76.8	76.8	64
Exposure Time (s)	10	5	5	5	5	5	6	6	6	12	12	12	10
Number of Images	3925	7858+15288	7858+15288	7858+15289	7858+15290	7858+15291	3194	3194	3194	700	700	700	885
Number of Frames/Image	80	100	100	100	100	100	120	120	120	60	60	60	50
Symmetry imposed	C4	C1	C1	C1	C1	C1	C1	C1	C1	C4	C4	C4	C4
Initial Particle Number	1,111,567	1,803,896+6,768,852	1,803,896+6,768,852	1,803,896+6,768,852	1,803,896+6,768,852	1,803,896+6,768,852	925,366	925,366	925,366	175,735	175,735	175,735	265,119
Final Particle Number	97,601	264,391	108,641	39,680	235,666	44,530	26,158	40,532	56,646	41,166	11,276	21,512	39,056
Map resolution (Å)	2.6	2.71	3.03	3.3	3.08	3.47	3.16	3.64	3.26	3.7	3.89	3.66	3.64
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
Refinement													
Initial model used (PDB code)	3J5P	3J5P	n/a	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P
Model resolution (Å)	2.7	2.8	n/a	3.4	3.1	3.5	3.2	3.7	3.3	3.8	4.1	3.8	3.8
FSC threshold	0.5	0.5	n/a	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Map sharpening *B* factor ($Å^2$)	-92	-104	n/a	-98	-124	-114	-97	-126	-101	-157	-148	-153	-160
Model composition													
Non-hydrogen atoms	18452	20583	n/a	21381	20171	18821	17741	17594	17749	17436	16002	17340	15800
Protein residues	2234	2506	n/a	2611	2463	2286	2144	2144	2144	2124	1976	2116	1920
water	0	0	n/a	4	4	10	4	0	0	0	0	0	0
Ligands	9	9	n/a	9	9	10	9	6	9	4	0	4	4
B factors ($Å^2$)													
Protein	69	89	n/a	95	55	46	106	124	108	102	132	101	120
Ligand	51	57	n/a	70	32	32	77	88	78	73	n/a	64	61
water	n/a	n/a	n/a	67	18	11	74	n/a	n/a	n/a	n/a	n/a	n/a
R.m.s. deviations													
Bond lengths (Å)	0.006	0.007	n/a	0.008	0.005	0.005	0.015	0.013	0.015	0.012	0.008	0.008	0.015
Bond angles (°)	1.168	1.199	n/a	1.199	1.069	1.051	1.064	1.247	1.332	1.207	1.129	1.073	1.337
Validation													
MolProbity score	1.32	1.17	n/a	1.14	1.09	1.09	1.35	1.33	1.34	1.45	1.48	1.34	1.66
Clashscore	1.42	0.88	n/a	0.61	0.67	0.83	1.9	2.56	2.13	2.01	2.61	2.22	3.14
Poor rotamers (%)	0.35	0.32	n/a	0.35	0.6	0.15	0.42	0.37	0.79	0.75	0.46	0.16	1.87
Ramachandran plot													
Favored (%)	93.17	93.92	n/a	93.31	94.53	95.19	94.05	95.79	94.94	92.17	93.24	95.14	95.01
Allowed (%)	6.69	6.04	n/a	6.62	5.47	4.68	5.95	4.21	5.01	7.83	6.56	4.86	4.99
Disallowed (%)	0.13	0.04	n/a	0.08	0	0.13	0	0	0.05	0	0.2	0	0

	miniV1 in complex with RTX when QX-314 added under low Na^+ concentration						miniV1 in complex with RTX when YO-PRO-1 added under low Na^+ concentration		FLV1_apo (EMD-23128) (PDB-7L2H)	FLV1_RTX C_1 (EMD-23134) (PDB-7L2N)	FLV1_RTX O_1 (EMD-23132) (PDB-7L2L)	FLV1_RTX C_2 (EMD-24083) (PDB-7MZ5)	FLV1_DkTx-RTX (EMD-23133) (PDB-7L2M)
	1 perturbed PI (EMD-24084) (PDB-7MZ6)	4 partially bound RTX (EMD-24085) (PDB-7MZ7)	1 partially bound RTX (EMD-24086) (PDB-7MZ9)	2 bound RTX in adjacent pockets (EMD-24087) (PDB-7MZA)	2 bound RTX in opposite pockets (EMD-24091) (PDB-7MZE)	3 bound RTX and 1 perturbed PI (EMD-24088) (PDB-7MZB)	4 fully engaged RTX C_1 (EMD-24089) (PDB-7MZC)	4 fully engaged RTX C_2 (EMD-24090) (PDB-7MZD)					
Data Collection/Processing													
Microscope	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Talos Arctica
Detector	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K2	Gatan K2	Gatan K2	Gatan K2	Gatan K3
Voltage (kV)	300	300	300	300	300	300	300	300	300	300	300	300	200
Magnification	105,000	105,000	105,000	105,000	105,000	105,000	105,000	105,000	29,000	130,000	130,000	130,000	36,000
Defocus Range (μm)	-0.8 to -2.0	-0.8 to -2.0	-0.8 to -2.0	-0.8 to -2.0	-0.8 to -2.0	-0.8 to -2.0	-0.8 to -2.0	-0.8 to -2.0	-1.0 to -2.0	-1.0 to -2.0	-1.0 to -2.0	-1.0 to -2.0	-0.5 to -3.0
Pixel Size (Å)	0.834	0.834	0.834	0.834	0.834	0.834	0.834	0.834	0.822	1.032	1.032	1.032	1.14
Total Electron Dose ($e^-/Å^2$)	66	66	66	66	66	66	66	66	74	70.4	70.4	70.4	55.4
Exposure Time (s)	6	6	6	6	6	6	6	6	10	10	10	10	2.4
Number of Images	2427	2427	2427	2427	2427	2427	2783	2783	4022	4109	4109	4109	1174
Number of Frames/Image	120	120	120	120	120	120	120	120	100	50	50	50	120
Symmetry imposed	C1	C1	C1	C1	C1	C1	C4	C4	C4	C4	C4	C4	C4
Initial Particle Number	2,160,229	2,160,229	2,160,229	2,160,229	2,160,229	2,160,229	2,361,933	2,361,933	2,014,065	3,409,673	3,409,673	3,409,673	818,141
Final Particle Number	61,712	23,122	38,184	19,877	10,873	18,122	30,401	42,542	151,505	24,855	29,314	81,361	36,689
Map resolution (Å)	2.91	3.35	3.18	3.46	3.42	3.72	3.03	2.9	2.63	3.09	3.42	2.76	3.84
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
Refinement													
Initial model used (PDB code)	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P	3J5P
Model resolution (Å)	3	3.5	3.2	3.6	3.6	3.8	3.2	2.9	2.7	3.3	3.7	2.9	4
FSC threshold	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Map sharpening *B* factor ($Å^2$)	90	105	85	86	82	98	99	91	-94	-99	-136	-99	-176
Model composition													
Non-hydrogen atoms	20381	17893	18644	18246	17622	17611	17338	17490	17560	14728	14256	14295	15985
Protein residues	2476	2180	2278	2229	2147	2145	2112	2128	2123	1780	1716	1724	1934
water	0	0	0	0	0	0	0	4	0	0	0	0	0
Ligands	9	5	5	5	5	5	6	6	9	4	4	7	7
B factors ($Å^2$)													
Protein	84	109	104	123	107	134	102	88	80	97	98	77	105
Ligand	63	83	78	102	85	112	65	58	48	70	65	41	85
water	n/a	n/a	n/a	n/a	n/a	n/a	n/a	51	n/a	n/a	n/a	n/a	n/a
R.m.s. deviations													
Bond lengths (Å)	0.009	0.012	0.01	0.009	0.009	0.01	0.01	0.007	0.005	0.008	0.015	0.013	0.007
Bond angles (°)	1.116	1.281	1.14	1.152	1.166	1.204	1.22	1.171	1.067	1.21	1.404	1.196	1.158
Validation													
MolProbity score	1.22	1.27	1.25	1.32	1.31	1.32	1.28	1.02	1.16	1.25	1.52	1.06	1.3
Clashscore	1.16	1.4	1.23	1.7	1.59	1.65	1.62	0.8	0.89	1.56	3.4	0.77	1.79
Poor rotamers (%)	0.55	0.41	0.45	0.66	0.42	0.84	0.96	0.59	0.53	0.25	0.06	0.77	0.75
Ramachandran plot													
Favored (%)	94.25	94.11	93.96	94.01	93.92	94.01	94.58	96.04	94.28	94.9	94.33	95.47	94.67
Allowed (%)	5.51	5.71	5.81	5.99	5.84	5.9	5.28	3.96	5.72	5.1	5.67	4.12	5.33
Disallowed (%)	0.24	0.18	0.22	0	0.23	0.09	0.14	0	0	0	0	0.41	0