

**Supporting Information for**

Structural insight into sodium ion pathway in the bacterial flagellar stator from marine *Vibrio*.

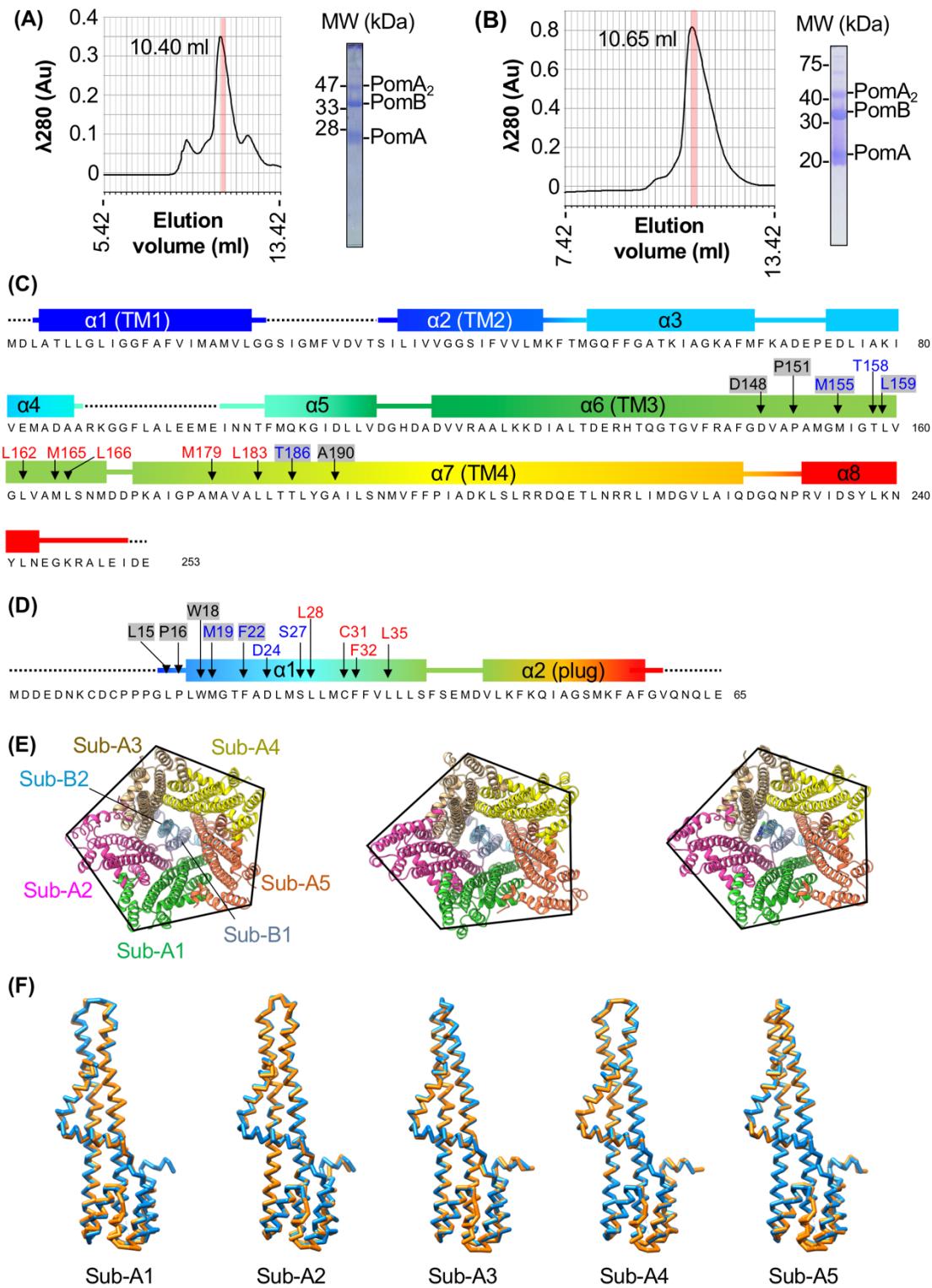
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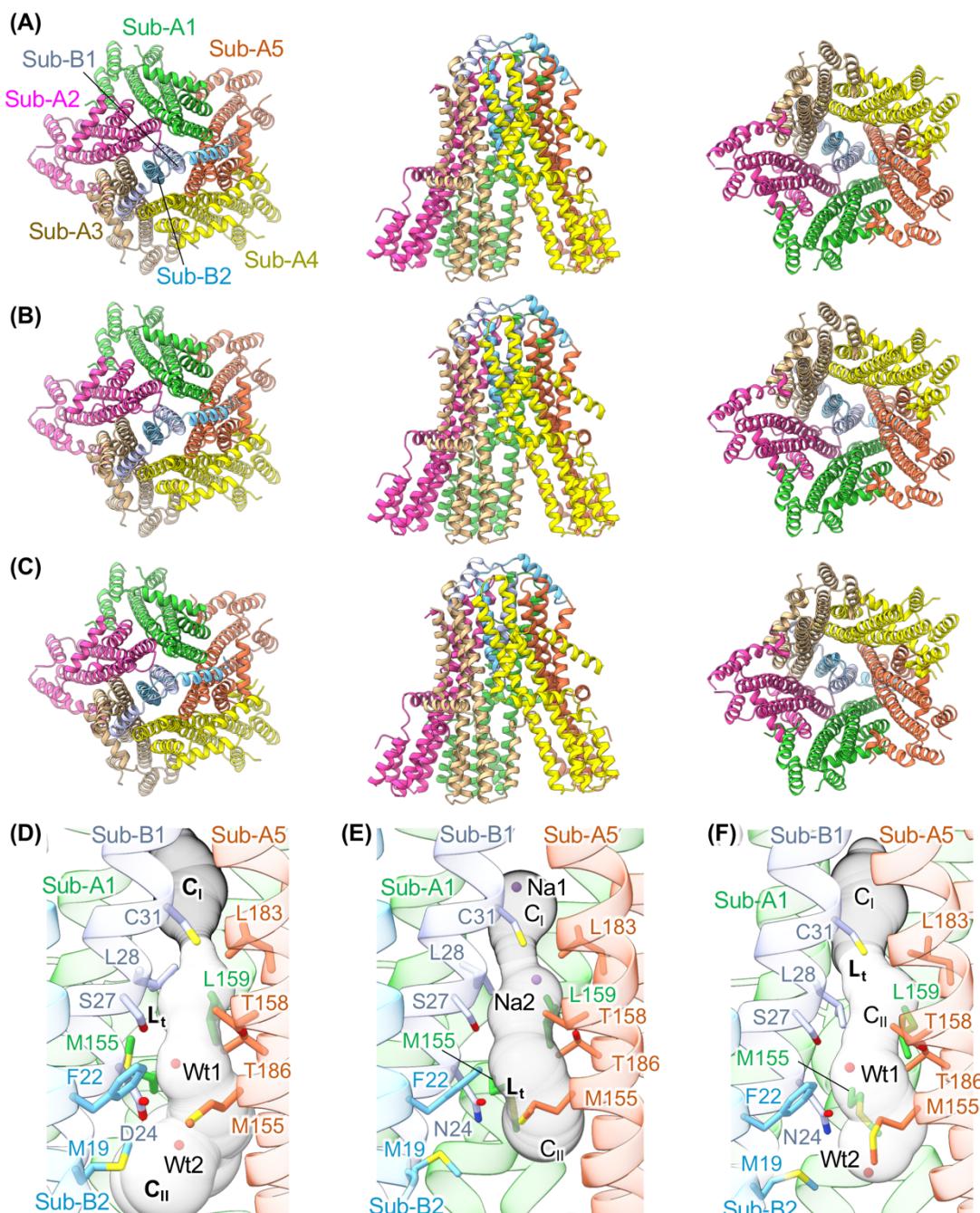
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**Fig. S1. Structure analysis of Va-PomAB.** (A) (B) SEC elution profile and SDS-PAGE pattern of Va-PomAB (A) and Va-PomAB(D24N) (B) samples with LMNG and 100 mM NaCl. The fractions shown by red boxes in the SEC profiles were examined by SDS-PAGE and used for cryo-EM structure analysis. (C) (D) Secondly structure and amino acid sequence of Va-PomA (C) and the

N-terminal region of Va-PomB (D). Residues not included in the model are shown as black dot lines. Residues that form Cavity I and II are indicated by red and blue letters, respectively. Phenamil binding sites are shown by letters with a gray shade. (E) Comparison of the subunit arrangement of Va-PomAB with 100 mM NaCl (left), Va-PomAB with 300 mM NaCl (middle) (PDB code: 8BRD), and Va-PomAB with phenamil (right). Ribbon models viewed from the cytoplasm are drawn. The subunits are colored as in Fig. 1D. The C $\alpha$  atoms of A86 (the C-terminus of  $\alpha$ 4) in the neighboring subunits are linked. (F) Structural comparison of PomA subunits at equivalent positions in the PomA ring of Va-PomAB with 100 mM NaCl and with 300 mM NaCl (PDB code: 8BRD). C $\alpha$  traces of each PomA of Va-PomAB with 100 mM NaCl (cyan) is superimposed on that of 8BRD (orange).



**Fig. S2. Structures of Va-PomAB at 100 mM KCl and Va-PomAB (D24N) at 100 mM NaCl and at 100 mM KCl.** Ribbon representation of (A) Va-PomAB at 100 mM KCl, (B) Va-PomAB (D24N) at 100 mM NaCl, and (C) Va-PomAB (D24N) at 100 mM KCl viewed from periplasm (left), side (middle), and cytoplasm (right). Close-up side view of the long tunnel ( $L_t$ ) of (D) Va-PomAB at 100 mM KCl, (E) Va-PomAB (D24N) at 100 mM NaCl, and (F) Va-PomAB (D24N) at 100 mM KCl. The residues that form the inner wall of the long tunnel are shown by the stick model. Sodium ions (Na1 and Na2) and solvent molecules (Wt1 and Wt2) in the long tunnel are indicated by balls. Cavity-I ( $C_I$ ) and Cavity-II ( $C_{II}$ ) are indicated by dark and light gray blobs, respectively. The subunits are colored as in Fig. 1D.

	<b>a1 (TM1)</b>	<b>a2 (TM2)</b>	<b>a3</b>	
VaPomA	----MDLATLGLIGGF <sup>A</sup> FVIMAMVLGG----S <sup>B</sup> IGMFVDVTSILIVVGGSI <sup>C</sup> FVVL <sup>D</sup> MKF <sup>E</sup> TMQFFF <sup>F</sup> GATK <sup>G</sup> IAGKAFM <sup>H</sup> -FK--AD			71
VmPomA	--MDLATLIGLIGGLAFVIMAMVLGG--SLMMFVDDVSILIVVGGSVFVVL <sup>D</sup> MKF <sup>E</sup> TMQFFF <sup>F</sup> GAGK <sup>G</sup> IASKAFM <sup>H</sup> -FK--AD			71
VcPomA	--MDLATLVGLIGGM <sup>A</sup> FVIMAMVLGG--S <sup>B</sup> IMMFVDDVSILIVVGGSVFVVL <sup>D</sup> MKF <sup>E</sup> EMGQFFF <sup>G</sup> AAK <sup>H</sup> IAGKAFM <sup>I</sup> -FK--AD			71
VpPomA	--MDLATLIGLIGGF <sup>A</sup> FVIMAMVLGG--S <sup>B</sup> IGMFVDVTSILIVVGGSAF <sup>C</sup> VVL <sup>D</sup> MKF <sup>E</sup> LGQFFGAAK <sup>G</sup> IAGKAFM <sup>H</sup> -FK--AD			71
SoPomA	--MDLATIIGLVGSFGFI <sup>A</sup> WSMV <sup>B</sup> ISG----GVMMFYD <sup>C</sup> LASVVIVF <sup>D</sup> GGSSFFVVM <sup>E</sup> MKF <sup>F</sup> NLKQFLGAVK <sup>G</sup> IAAKAFI <sup>H</sup> -FK--ID			71
BsMotP	MKRF <sup>A</sup> DYLTPVG <sup>B</sup> FVLGTIIIVIGIISGSGVSGFRSF <sup>C</sup> LDLTSFF <sup>D</sup> ITV <sup>E</sup> GLCAAVF <sup>F</sup> ISFPPSELKKAPS <sup>G</sup> VLKQAFI <sup>H</sup> -RQ--ED			77
RsMotA	--MDIAAAIGLIGAIVMVG <sup>B</sup> GS <sup>C</sup> YIAG----GVAPF <sup>D</sup> V <sup>E</sup> PLSLIV <sup>F</sup> IVVAGTAF <sup>G</sup> I <sup>H</sup> VLAMK <sup>D</sup> P <sup>E</sup> VLF <sup>G</sup> HFKAMMKV <sup>H</sup> K-PS--RF			71
BsMotA	--MDKTSLIGIILAFVALS <sup>A</sup> VGVMVLKGV--SF <sup>B</sup> SLANPA <sup>C</sup> AII <sup>D</sup> IIAGTISAVVIAF <sup>E</sup> PTKEIKV <sup>F</sup> PALF <sup>G</sup> RVL <sup>H</sup> KENK--QL			73
CjMotA	--MDLSTI <sup>A</sup> LGMLVALTS <sup>B</sup> VGDI <sup>C</sup> LEGG--NPLHV <sup>D</sup> IHLSS <sup>E</sup> LF <sup>F</sup> IVMPTAA <sup>G</sup> C <sup>H</sup> AMTSHKK <sup>I</sup> VKAA <sup>J</sup> YKELKV <sup>K</sup> VFK-GS--GV			72
EcMotA	--MLILLG <sup>A</sup> LYLV <sup>B</sup> VLGTVFGGYLM <sup>C</sup> TGG--SLGALYQ <sup>D</sup> PAELV <sup>E</sup> IIAGAG <sup>F</sup> IGS <sup>G</sup> IVGNNGKA <sup>H</sup> IKGTL <sup>I</sup> KALPLL <sup>J</sup> FR <sup>K</sup> RSKYTKA			73
SeMotA	--MLILLG <sup>A</sup> LYLV <sup>B</sup> IGTVFGGYVM <sup>C</sup> TGG--HLGALYQ <sup>D</sup> PAELV <sup>E</sup> II <sup>F</sup> GGAG <sup>G</sup> IGAF <sup>H</sup> IVGNNGKA <sup>I</sup> KGTMKA <sup>J</sup> IP <sup>K</sup> LL <sup>L</sup> FR <sup>M</sup> RSKYTKS			73
	<b>a4</b>	<b>a5</b>		
VaPomA	EPEDLIAK <sup>A</sup> IVE <sup>B</sup> MADA <sup>C</sup> ARKGGF <sup>D</sup> LA <sup>E</sup> LEM--E <sup>F</sup> IN-----NTFMQKG <sup>G</sup> IDLLV <sup>H</sup> DGHAD-VVRAALKKDIAL			131
VmPomA	EPEDLIAK <sup>A</sup> IVE <sup>B</sup> MADA <sup>C</sup> ARKGGF <sup>D</sup> LA <sup>E</sup> LEM--E <sup>F</sup> IP-----NPFMQKG <sup>G</sup> IDLLV <sup>H</sup> DGHAD-VV <sup>I</sup> RATLQKDIAL			131
VcPomA	APEDLIAK <sup>A</sup> IVE <sup>B</sup> MADA <sup>C</sup> ARKGGF <sup>D</sup> LA <sup>E</sup> LEM--E <sup>F</sup> IP-----NPFMQKG <sup>G</sup> IDLLV <sup>H</sup> DGHAD-VV <sup>I</sup> RATLQKDIAL			131
VpPomA	EPEDLIAK <sup>A</sup> IVE <sup>B</sup> MADA <sup>C</sup> ARKGGF <sup>D</sup> LA <sup>E</sup> LEM--E <sup>F</sup> IN-----NSFMQKG <sup>G</sup> IDLLV <sup>H</sup> DGHAD-VVRAALQKDIAL			131
SoPomA	RPEDLIEQS <sup>A</sup> VTMADA <sup>B</sup> ARKGGF <sup>C</sup> LA <sup>D</sup> EEA---Q <sup>E</sup> IS-----NSFMQKAVDMLV <sup>H</sup> DGHGE-VVRAALEKDITL			131
BsMotP	NVKDVL <sup>A</sup> KTFV <sup>B</sup> SLSLDHARKH <sup>C</sup> GLLSLDDQAREI <sup>D</sup> K-----DPFLKKG <sup>E</sup> LLAIDGWDEE-TIRLVM <sup>F</sup> DSEIAA			139
RsMotA	DMNEVISTMVELSNLARKDG <sup>A</sup> IMALEGK--AVP-----DAFFEKG <sup>B</sup> LQLLV <sup>C</sup> DGTDEA-KLV <sup>D</sup> Q <sup>E</sup> LYE <sup>F</sup> IK <sup>G</sup> A			131
BsMotA	TIEELIPM <sup>A</sup> SEWAQLARREG <sup>B</sup> LLA <sup>C</sup> ES <sup>D</sup> IEDV <sup>E</sup> D-----DAFLKNGL <sup>F</sup> MSMA <sup>G</sup> VDGQSAE-FIRD <sup>H</sup> IMTEEEVA			135
CjMotA	NLPERIAQ <sup>A</sup> LIEFA <sup>B</sup> IIARRDGL <sup>C</sup> LAESRTNE <sup>D</sup> E-----NEFLKNAMMML <sup>E</sup> VDGK <sup>F</sup> SE-EIHESME <sup>G</sup> IQTEQ			134
EcMotA	MYMDLL <sup>A</sup> LALLYRLMAKS <sup>B</sup> RQGMFS <sup>C</sup> LERD <sup>D</sup> ENPRESEI <sup>E</sup> FA <sup>F</sup> SYPR <sup>G</sup> ILADSVMLDF <sup>H</sup> IVDYLRL <sup>I</sup> ISGHMNT <sup>J</sup> FEIEALMDEE <sup>K</sup> E <sup>L</sup> T			153
SeMotA	MYMDLL <sup>A</sup> LALLYRLMAKS <sup>B</sup> RQGMFS <sup>C</sup> LERD <sup>D</sup> ENPRESEI <sup>E</sup> FA <sup>F</sup> SYPR <sup>G</sup> ILADSVMLDF <sup>H</sup> IVDYLRL <sup>I</sup> ISGNMNT <sup>J</sup> FEIEALMDEE <sup>K</sup> E <sup>L</sup> T			153
	<b>a6 (TM3)</b>	<b>a7 (TM4)</b>		
VaPomA	TDERHTQGTGV <sup>A</sup> FR <sup>B</sup> FGDV <sup>C</sup> APAMGM <sup>D</sup> IGTLV <sup>E</sup> GLV <sup>F</sup> AML <sup>G</sup> SNMDDPK-A <sup>H</sup> IGPAMAVALLTTLYGAI <sup>I</sup> LSNMV <sup>J</sup> FFPIADKL <sup>K</sup> SLRRDQ			210
VmPomA	TDERHSKG <sup>A</sup> TQV <sup>B</sup> FR <sup>C</sup> FGDV <sup>D</sup> APAMGM <sup>E</sup> IGTLV <sup>F</sup> GLV <sup>G</sup> AML <sup>H</sup> SNMDDPK-S <sup>I</sup> IGPAMAVALLTTLYGAV <sup>J</sup> LSN <sup>K</sup> IFFPIADKL <sup>L</sup> SLRRDQ			210
VcPomA	TDERHSKG <sup>A</sup> TQV <sup>B</sup> FR <sup>C</sup> FGDV <sup>D</sup> APAMGM <sup>E</sup> IGTLV <sup>F</sup> GLV <sup>G</sup> AML <sup>H</sup> SNMDDPK-S <sup>I</sup> IGPAMAVALLTTLYGAV <sup>J</sup> LSN <sup>K</sup> IFFPIADKL <sup>L</sup> SLRRDQ			210
VpPomA	TDERHTQGTGV <sup>A</sup> FR <sup>B</sup> FGDV <sup>C</sup> APAMGM <sup>D</sup> IGTLV <sup>E</sup> GLV <sup>F</sup> AML <sup>G</sup> SNMDDPK-A <sup>H</sup> IGPAMAVALLTTLYGAV <sup>I</sup> LSN <sup>J</sup> MLFFPIADKL <sup>K</sup> SLRRDQ			210
SoPomA	TEDRHRIGIA <sup>A</sup> IFRAFADVG <sup>B</sup> PAMGM <sup>C</sup> IGTLV <sup>D</sup> GLV <sup>E</sup> AML <sup>F</sup> NSMDDPK-S <sup>G</sup> IGPSMA <sup>H</sup> VALLTTLYGAV <sup>I</sup> LAN <sup>J</sup> MCV <sup>K</sup> PIADKL <sup>L</sup> SLRMGE			210
BsMotP	MEERHRKGRR <sup>A</sup> FEKAGEF <sup>B</sup> APAWGM <sup>C</sup> IGTLV <sup>D</sup> GLV <sup>E</sup> MLKNLNDPK-M <sup>G</sup> LPNMA <sup>H</sup> IALLTTLYG <sup>I</sup> SLLANMV <sup>J</sup> FNPIAAKLEEKTES			218
RsMotA	MKARHEAYQGAVKAW <sup>A</sup> ID <sup>B</sup> GPAMGMV <sup>C</sup> GT <sup>D</sup> IGLVLMLGNMS <sup>E</sup> MLDPM <sup>F</sup> PI <sup>G</sup> IGPAMAVALLTTLYG <sup>H</sup> ALMANV <sup>I</sup> F <sup>J</sup> APILNKLEGYSAD			210
BsMotA	MEDRHQAGAA <sup>A</sup> IFTQAGTY <sup>B</sup> AP <sup>C</sup> TLG <sup>D</sup> VLGAV <sup>E</sup> IGL <sup>F</sup> IA <sup>G</sup> ALSHMDNTD-ELGA <sup>H</sup> IA <sup>I</sup> SAF <sup>J</sup> VAT <sup>K</sup> LLGIFT <sup>L</sup> GYV <sup>M</sup> WHPF <sup>N</sup> ANKL <sup>O</sup> KRSKQ <sup>P</sup>			214
CjMotA	LEEHYKECAEYW <sup>A</sup> IV <sup>B</sup> FGETC <sup>C</sup> PTM <sup>D</sup> GLV <sup>E</sup> GA <sup>F</sup> V <sup>G</sup> FL <sup>H</sup> ILA <sup>I</sup> LAKL <sup>J</sup> LDNPQ-AMAAG <sup>K</sup> ISGAFTA <sup>L</sup> T <sup>M</sup> V <sup>N</sup> TG <sup>P</sup> IF <sup>R</sup> GYA <sup>S</sup> LF <sup>T</sup> APWGKKL <sup>U</sup> KANGMD			213
EcMotA	HESEAEVP <sup>A</sup> ANS <sup>B</sup> LA <sup>C</sup> VGD <sup>D</sup> SLPAFG <sup>E</sup> IV <sup>F</sup> AV <sup>G</sup> MGV <sup>H</sup> HAL <sup>I</sup> ASDR <sup>J</sup> AA <sup>K</sup> ELG <sup>L</sup> ALIAHAMVG <sup>M</sup> TF <sup>N</sup> LG <sup>P</sup> LLAYGF <sup>R</sup> ISPLAT <sup>S</sup> VLRQ <sup>T</sup> KSAE			233
SeMotA	HESEAEVP <sup>A</sup> ANS <sup>B</sup> LA <sup>C</sup> VGD <sup>D</sup> SLPAFG <sup>E</sup> IV <sup>F</sup> AV <sup>G</sup> MGV <sup>H</sup> HAL <sup>I</sup> ASDR <sup>J</sup> AA <sup>K</sup> ELG <sup>L</sup> ALIAHAMVG <sup>M</sup> TF <sup>N</sup> LG <sup>P</sup> LLAYGF <sup>R</sup> ISPLAT <sup>S</sup> VLRQ <sup>T</sup> KSAE			233
	<b>a8</b>			
VaPomA	ETLNRR <sup>A</sup> LI <sup>B</sup> MDGVL <sup>C</sup> A <sup>D</sup> QDGQNPR <sup>E</sup> VID <sup>F</sup> SYL <sup>G</sup> KNL <sup>H</sup> NEGKRAL-E <sup>I</sup> IDE-----			253
VmPomA	ETLNRR <sup>A</sup> LI <sup>B</sup> MDGVL <sup>C</sup> A <sup>D</sup> QDGQNPR <sup>E</sup> VID <sup>F</sup> SYL <sup>G</sup> KNL <sup>H</sup> NEGKRAL-D <sup>I</sup> DVLK <sup>J</sup> KE-----			254
VcPomA	ETLNRR <sup>A</sup> LI <sup>B</sup> MDGVL <sup>C</sup> A <sup>D</sup> QDGQNPR <sup>E</sup> VID <sup>F</sup> SYL <sup>G</sup> KNL <sup>H</sup> NEGKRAL-D <sup>I</sup> DVLK <sup>J</sup> KE-----			254
VpPomA	ETLNRR <sup>A</sup> LI <sup>B</sup> MDGVL <sup>C</sup> A <sup>D</sup> QDGQNPR <sup>E</sup> VID <sup>F</sup> SYL <sup>G</sup> KNL <sup>H</sup> NEGKRAL-E <sup>I</sup> IDE-----			253
SoPomA	EMLNRNL <sup>A</sup> IMDAVL <sup>B</sup> A <sup>C</sup> QDGQNPR <sup>D</sup> VI <sup>E</sup> EGFL <sup>F</sup> KNL <sup>G</sup> AEKQR <sup>H</sup> KI-D <sup>I</sup> TTTDGE-----			255
BsMotP	E <sup>A</sup> I <sup>B</sup> IKQVMVEGI <sup>C</sup> IGVQSG <sup>D</sup> KNPR <sup>E</sup> N <sup>F</sup> SQLV <sup>G</sup> V <sup>H</sup> SSREEWQK-Q <sup>I</sup> KPVQV <sup>J</sup> TKKG <sup>K</sup> S-----H-EA--			272
RsMotA	EV <sup>A</sup> TYREL <sup>B</sup> VI <sup>C</sup> EGLRG <sup>D</sup> I <sup>E</sup> ARGES <sup>F</sup> ARMEDQMV <sup>G</sup> CALDRKQ <sup>H</sup> QMK-RKAA-----			253
BsMotA	EV <sup>A</sup> VL <sup>B</sup> REV <sup>C</sup> ML <sup>D</sup> IGV <sup>E</sup> LSV <sup>F</sup> LGQ <sup>G</sup> Q <sup>H</sup> PKV <sup>I</sup> E <sup>J</sup> Q <sup>K</sup> L <sup>L</sup> MLP <sup>M</sup> AKDRL-K <sup>N</sup> FAEQEQAQN <sup>P</sup> GEK--K--EEE <sup>R</sup> A			270
CjMotA	L <sup>A</sup> VKEQ <sup>B</sup> IV <sup>C</sup> TE <sup>D</sup> AKG <sup>E</sup> IA <sup>F</sup> EGAN <sup>G</sup> PRD <sup>H</sup> LEAKL <sup>I</sup> FLN <sup>J</sup> SHD <sup>K</sup> DP <sup>L</sup> R <sup>M</sup> ISQFD <sup>N</sup> KG-----			258
EcMotA	TSKMMQC <sup>A</sup> V <sup>B</sup> KV <sup>C</sup> TL <sup>D</sup> LSN <sup>E</sup> LN <sup>F</sup> GYAPP <sup>G</sup> I <sup>H</sup> AVE <sup>I</sup> FGR <sup>J</sup> K <sup>K</sup> TLYSS <sup>M</sup> ERPS-F <sup>N</sup> IELEHHVRA <sup>P</sup> V <sup>Q</sup> KNPQQQT <sup>R</sup> TEE <sup>S</sup> A			295
SeMotA	TTKMMQC <sup>A</sup> V <sup>B</sup> K <sup>C</sup> TL <sup>D</sup> LSN <sup>E</sup> LN <sup>F</sup> GYAPP <sup>G</sup> I <sup>H</sup> AVE <sup>I</sup> FGR <sup>J</sup> K <sup>K</sup> TLYSS <sup>M</sup> ERPS-F <sup>N</sup> IELEHHVRA <sup>P</sup> V <sup>Q</sup> KNPQQQT <sup>R</sup> TEE <sup>S</sup> A			295
	<b>a2 (plug)</b>			
VaPomB	-----M----DDEDNKCD <sup>A</sup> CPPPG <sup>B</sup> PL <sup>C</sup> WLMGTF <sup>D</sup> ADLMS <sup>E</sup> LLMCFF <sup>F</sup> V <sup>G</sup> LLS <sup>H</sup> FSEMDVL <sup>I</sup> KFKQ <sup>J</sup> IAGSMKF <sup>K</sup> A <sup>L</sup> FGVQN			62
VmPomB	-----M----MDDEQQCKCP <sup>A</sup> PPPG <sup>B</sup> PL <sup>C</sup> WLMGTF <sup>D</sup> ADLMS <sup>E</sup> LLMCFF <sup>F</sup> V <sup>G</sup> LLS <sup>H</sup> FSEMDVL <sup>I</sup> KFKQ <sup>J</sup> IAGSMKF <sup>K</sup> A <sup>L</sup> FGVQN			61
VcPomB	-----M----MDDEQQCKCP <sup>A</sup> PPPG <sup>B</sup> PL <sup>C</sup> WLMGTF <sup>D</sup> ADLMS <sup>E</sup> LLMCFF <sup>F</sup> V <sup>G</sup> LLS <sup>H</sup> FSEMDVL <sup>I</sup> KFKQ <sup>J</sup> IAGSMKF <sup>K</sup> A <sup>L</sup> FGVQN			62
VpPomB	-----M----DDEDNKCD <sup>A</sup> CPPPG <sup>B</sup> PL <sup>C</sup> WLMGTF <sup>D</sup> ADLMS <sup>E</sup> LLMCFF <sup>F</sup> V <sup>G</sup> LLS <sup>H</sup> FSEMDVL <sup>I</sup> KFKQ <sup>J</sup> IAGSMKF <sup>K</sup> A <sup>L</sup> FGVQN			62
SoPomB	-----MAKCNC <sup>A</sup> CPPPG <sup>B</sup> PL <sup>C</sup> WLMGTF <sup>D</sup> ADLMS <sup>E</sup> LLMCFF <sup>F</sup> V <sup>G</sup> LLS <sup>H</sup> FSEMDVL <sup>I</sup> KFKQ <sup>J</sup> IAGSMKF <sup>K</sup> A <sup>L</sup> FGVQN			58
BsMotS	-----M <sup>A</sup> KLRRER--FERRNGSG <sup>B</sup> KNSQSSSSW <sup>C</sup> MVTFTD <sup>D</sup> L <sup>E</sup> TL <sup>F</sup> IL <sup>G</sup> V <sup>H</sup> F <sup>I</sup> LLF <sup>J</sup> MSQ <sup>K</sup> IDLQKF <sup>L</sup> KA <sup>M</sup> VDS <sup>N</sup> I <sup>O</sup> KEGNGLQ			68
RsMotB	-----MSAKPKV <sup>A</sup> IRFQP <sup>B</sup> V <sup>C</sup> PDD <sup>D</sup> GEDC <sup>E</sup> CP <sup>F</sup> CPPPG <sup>G</sup> APAWL <sup>H</sup> AT <sup>I</sup> FAD <sup>J</sup> T <sup>K</sup> NMAFF <sup>L</sup> V <sup>M</sup> I <sup>N</sup> GF <sup>P</sup> AKF <sup>Q</sup> DEPS <sup>R</sup> FSK <sup>S</sup> MAGAMRET <sup>T</sup> FGFHS			78
BsMotB	-----M----ARKKKKKHEDEHVDESWL <sup>A</sup> V <sup>B</sup> PYAD <sup>C</sup> ITLL <sup>D</sup> LA <sup>E</sup> F <sup>F</sup> IVLYASSSS <sup>G</sup> DAAKFQMLSKSF <sup>H</sup> NEVFTG <sup>I</sup> GGT			62
CjMotB	-----MAKKHKC <sup>A</sup> PEC <sup>B</sup> CP <sup>C</sup> AGEK <sup>D</sup> W <sup>E</sup> AV <sup>F</sup> PL <sup>G</sup> A <sup>H</sup> FL <sup>I</sup> SL <sup>J</sup> LA <sup>K</sup> W <sup>L</sup> IA <sup>M</sup> SKTNPA <sup>N</sup> VEALK <sup>P</sup> TEF <sup>R</sup> V <sup>S</sup> K <sup>T</sup> FDYTS			60
EcMotB	MKNQAHPI <sup>A</sup> IV <sup>B</sup> -----VKRR <sup>C</sup> KAKSH- <sup>D</sup> GAAGHGSW <sup>E</sup> KIAYADF <sup>F</sup> TAMMAFF <sup>G</sup> FLVMWL <sup>H</sup> IS <sup>I</sup> SSPKELI <sup>J</sup> QIAEY <sup>K</sup> FR <sup>L</sup> PLATAV			70
SeMotB	MKNQAHPI <sup>A</sup> IV <sup>B</sup> -----VKRR <sup>C</sup> RHKPH-GGGAGHGSW <sup>E</sup> KIAYADF <sup>F</sup> TAMMAFF <sup>G</sup> FLVMWL <sup>H</sup> IS <sup>I</sup> SSPKELI <sup>J</sup> QIAEY <sup>K</sup> FR <sup>L</sup> PLATAV			71

**Fig. S3. Amino acid sequence alignment of VaPomA and VaPomB homologs from different bacteria.** The secondary structure elements of VaPomA and B are shown above the sequence. Residues not included in the model are shown as black dot lines. Residues that form Cavity-I and II are indicated by red and blue letters, respectively. Phenamil binding sites are shown by letters with a gray shade. The aligned sequences are *Vibrio alginolyticus*, VaPomA and B; *Vibrio mimicus*, VmPomA and B; *Vibrio cholerae*, VcPomA and B; *V. parahaemolyticus*, VpPomA and B; *Shewanella oneidensis*, SoPomA and B; *Bacillus subtilis*, BsMotP, S, A, and B; *Rhodobacter sphaeroides*, RsMotA and B; *Campylobacter jejuni*, CjMotA and B; *Escherichia coli*, EcMotA and B; *Salmonella enterica*, SeMotA and B. VaPomAB, VmPomAB, VcPomAB, VpPomAB, SoPomAB, and BsMotPS are the sodium-driven stator, and RsMotAB, CjMotAB, EcMotAB, and SeMotAB are the proton-driven stator.

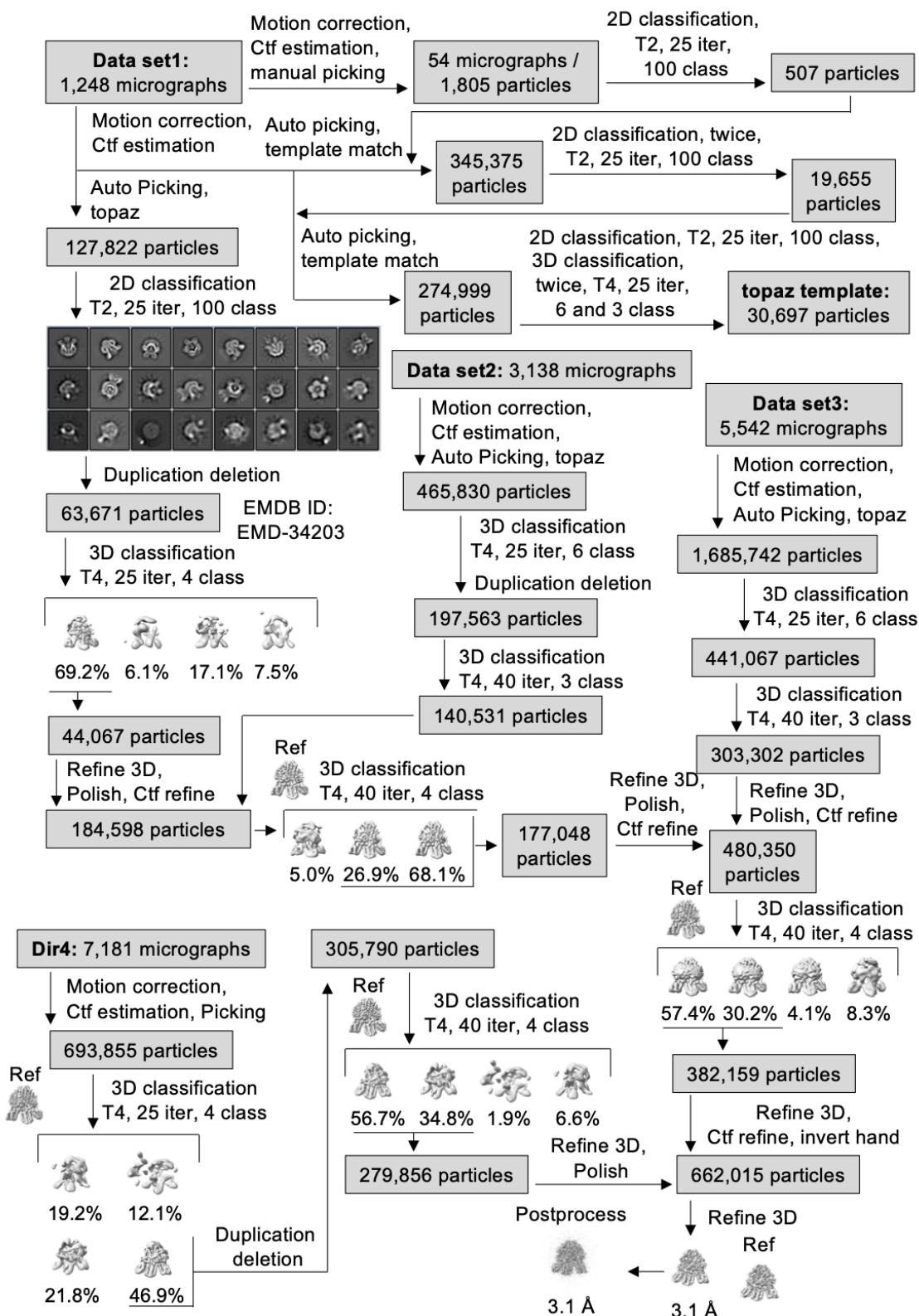


Fig. S4. 3D reconstruction scheme of Va-PomAB with NaCl.

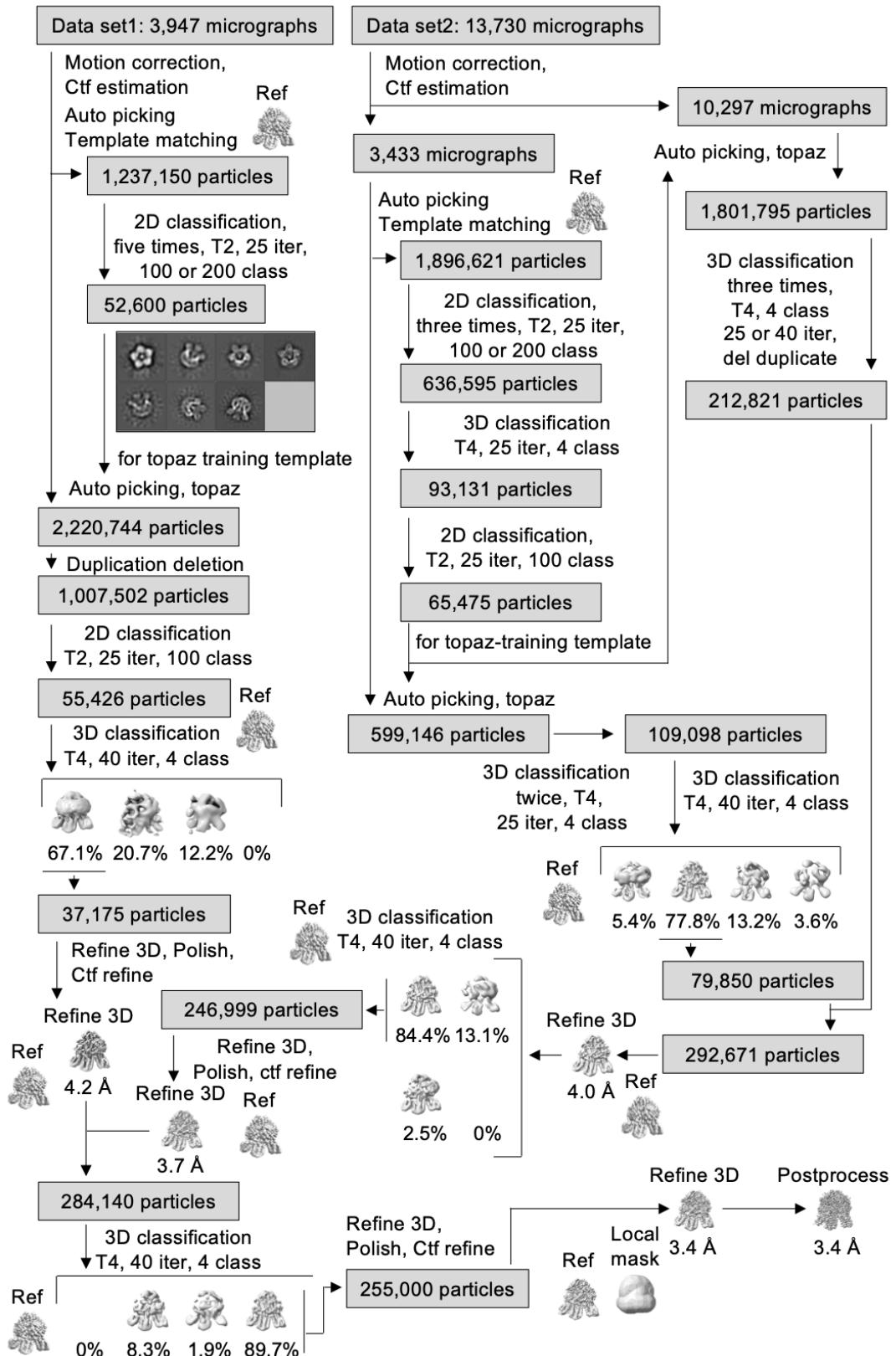


Fig. S5. 3D reconstruction scheme of Va-PomAB with KCl.

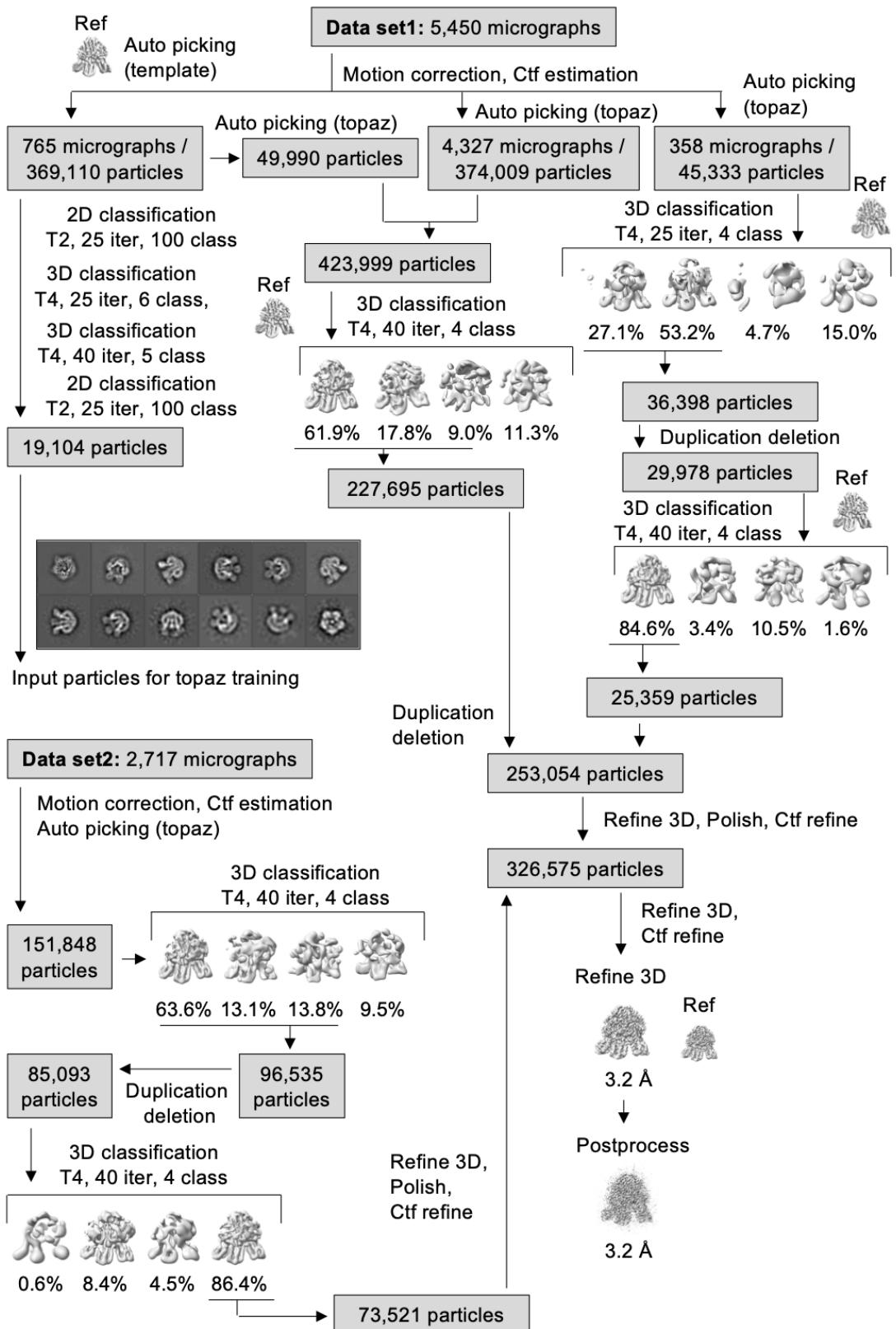
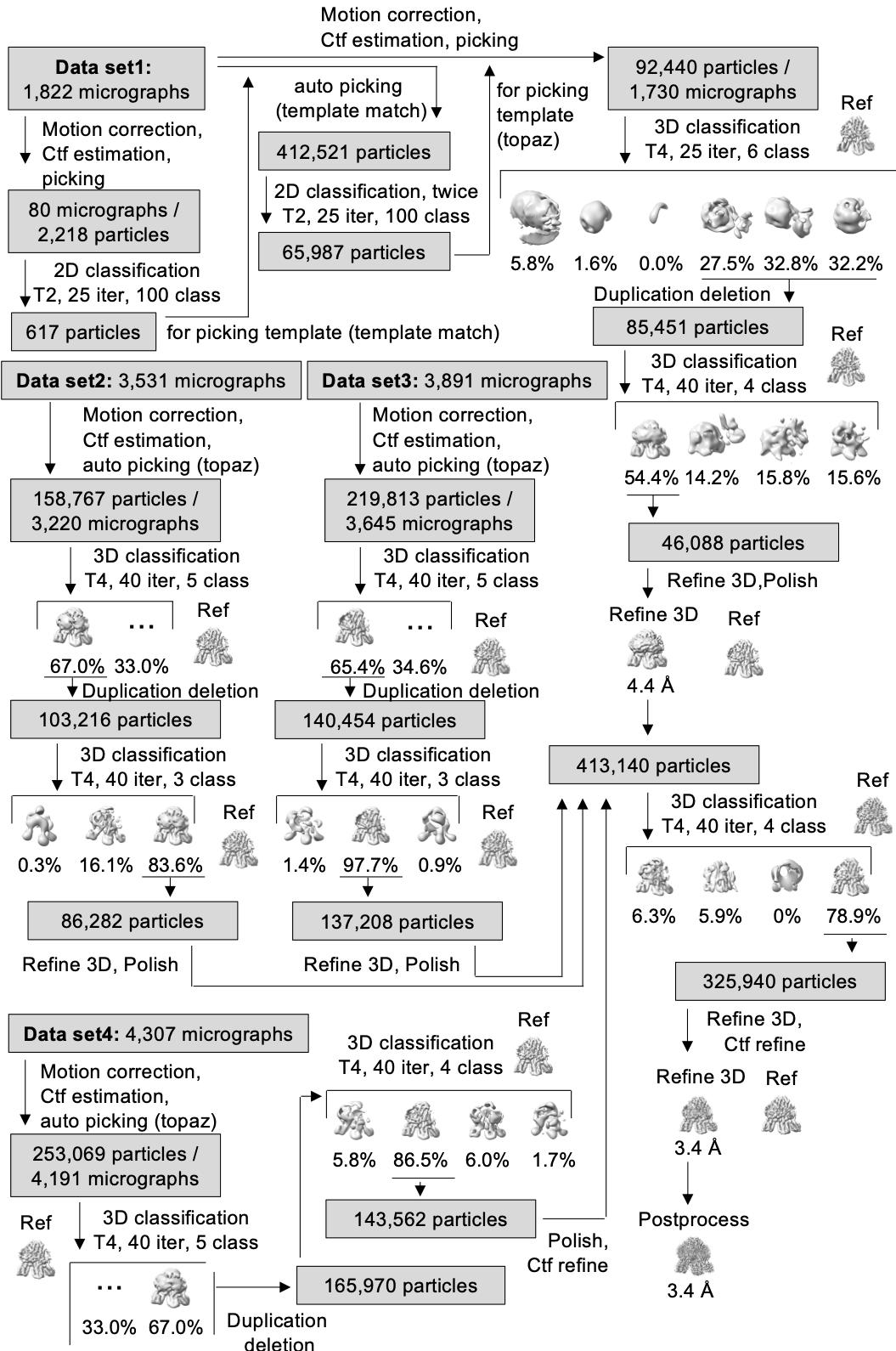


Fig. S6. 3D reconstruction scheme of Va-PomAB(D24N) with NaCl.



**Fig. S7. 3D reconstruction scheme of Va-PomAB(D24N) with KCl.**

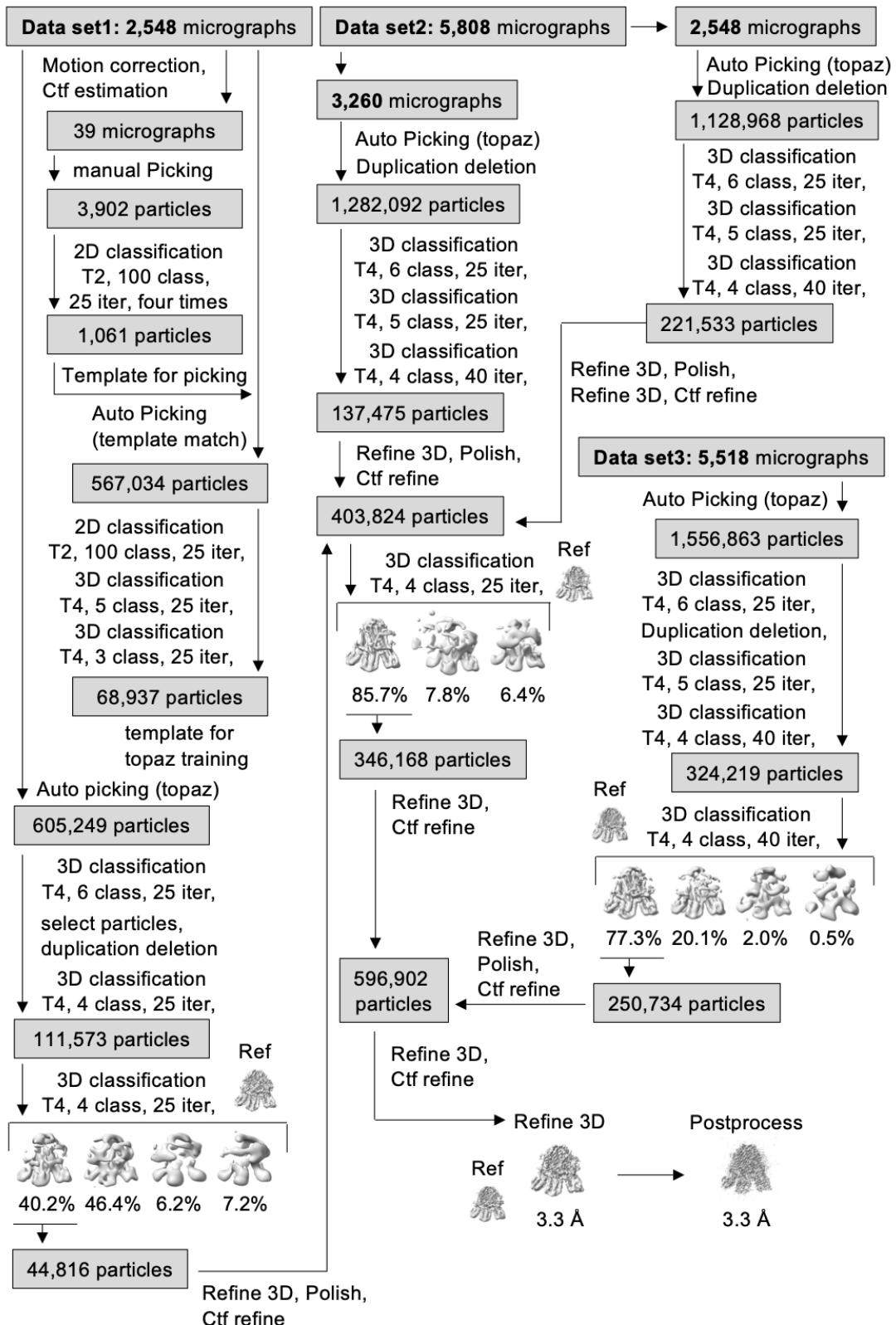
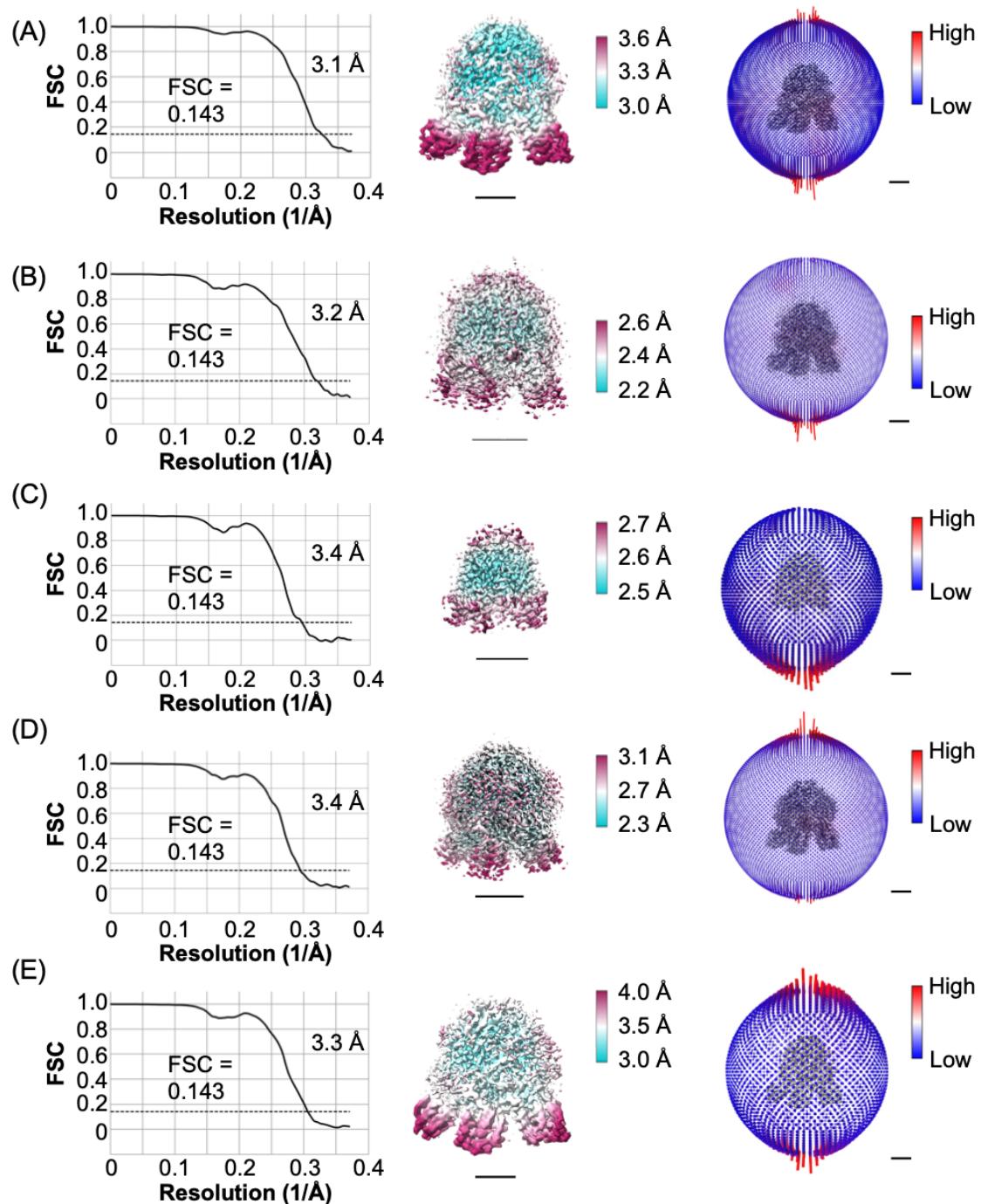


Fig. S8. 3D reconstruction scheme of Va-PomAB with Phenamil.



**Fig. S9. Resolution estimation of the cryo-EM map.** The Fourier shell correlation (FSC) curve, density map colored by local resolution, and angular distribution of particles used for 3D reconstruction are shown in the left, middle, and right panels, respectively. (A) Va-PomAB with NaCl, (B) Va-PomAB(D24N) with NaCl, (C) Va-PomAB with KCl, (D) Va-PomAB(D24N) with KCl, and (E) Va-PomAB with Phenamil.

**Table S1. Strains and plasmids used in this study**

<b>Strains or plasmids</b>	<b>Genotype or description</b>	<b>Reference or source</b>
<b><i>V. alginolyticus</i></b>		
VIO5	Wild type strain of a polar flagellum (Rif <sup>r</sup> Pof <sup>t</sup> Laf)	(1)
NMB191	VIO5 Δ <i>pomApomB</i> (Rif <sup>r</sup> Laf <sup>-</sup> Mot <sup>-</sup> )	(2)
<b><i>E. coli</i></b>		
DH5α	F <sup>-</sup> , Φ80d/ <i>lacZΔM15</i> , Δ( <i>lacZYA-argF</i> )U169, <i>deoR</i> , <i>recA1</i> , <i>endA1</i> , <i>hsdR17(rk<sup>-</sup>, m<sup>k+</sup>)</i> , <i>phoA</i> , <i>supE44</i> , λ <sup>-</sup> , <i>thi-1</i> , <i>gyrA96</i> , <i>relA1</i> (Host for cloning experiments)	(3)
BL21(DE3)/ pLysS	<i>F</i> <sup>-</sup> , <i>ompT</i> , <i>hsdSB</i> (r <sub>B</sub> <sup>-</sup> m <sub>B</sub> <sup>-</sup> ), <i>gal</i> (λ <sub>cI</sub> 857, <i>ind1</i> , <i>Sam7</i> , <i>nin5</i> , <i>lacUV5-T7gene1</i> ), <i>dcm</i> (DE3) (Host for protein expression)	Novagen
<b>Plasmids</b>		
pBAD33	Cm <sup>r</sup> , P <sub>BAD</sub>	(4)
pHFAB	<i>pomA</i> and <i>pomB</i> in pBAD33	(5)
pTSK37	<i>pomA</i> and <i>pomB</i> <sub>Δ41-120</sub> in pBAD33	(6)
pColdIV	Cold shock expression vector, Amp <sup>r</sup>	Takara
pCold4-pomAB-His <sub>6</sub>	<i>pomA</i> and <i>pomB-his</i> <sub>6</sub> in pColdIV	(7)

Rif<sup>r</sup>, rifampin resistant; Pof<sup>t</sup>, normal polar flagellum; Laf<sup>-</sup>, defective in lateral flagellar formation; Mot<sup>-</sup>, defective in polar flagellar motility; Cm<sup>r</sup>, chloramphenicol resistance; P<sub>BAD</sub>, arabinose promoter; Amp<sup>r</sup>, Ampicillin resistance.

**Table S2. Summary of data correction and model statistics.**

<b>Data collection and processing</b>	Va-PomAB 0.1 M NaCl (EMD-60580) (PDB 8ZYV)	Va-PomAB 0.1 M KCl (EMD-60581) (PDB 8ZYW)	Va-PomAB (D24N) 0.1 M NaCl (EMD-60584) (PDB 8ZZ)	Va-PomAB (D24N) 0.1 M KCl (EMD-60585) (PDB 8ZZ0)	Va-PomAB 0.1 M NaCl with phenamil (EMD-60636) (PDB 9IJM)
EM equipment	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios
Detector	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3
Energy filter	Gatan GIF Quantum, 20 eV slit	Gatan GIF Quantum, 20 eV slit	Gatan GIF Quantum, 20 eV slit	Gatan GIF Quantum, 20 eV slit	Gatan GIF Quantum, 20 eV slit
Ligand	-	-	-	-	0.1 mM Phenamil
Magnification	105,000	105,000	105,000	105,000	105,000
Voltage (kV)	300	300	300	300	300
Pixel size (Å)	0.675	0.675	0.675	0.675	0.675
Symmetry imposed	C1	C1	C1	C1	C1
<b>Data set1</b>					
Micrographs (no.)	1,248	3,947	5,450	1,822	2,548
Electron exposure (e <sup>-</sup> Å <sup>-2</sup> )	50	60	60	60	60
Defocus range (μm)	-1.8~0.8	-1.8~0.6	-1.8~0.6	-2.2~0.8	-2.0~0.725
Initial particle images (no.)	127,822	2,220,744	788,452	92,440	605,249
<b>Data set2</b>					
Micrographs (no.)	3,318	13,730	2,717	3,531	5,808
Electron exposure (e <sup>-</sup> Å <sup>-2</sup> )	60	60	60	60	60
Defocus range (μm)	-2.4~-1.125	-1.8~0.6	-1.8~0.6	-2.2~0.8	-2.8~0.8
Initial particle images (no.)	465,830	2,409,941	151,848	158,767	2,411,060
<b>Data set3</b>					
Micrographs (no.)	5542			3,891	5,518
Electron exposure (e <sup>-</sup> Å <sup>-2</sup> )	60	-	-	60	60
Defocus range (μm)	-2.4~-1.125			-2.2~0.8	-2.0~0.8
Initial particle images (no.)	1,865742			219,813	1,556,863
<b>Data set4</b>					
Micrographs (no.)	7,181			4,307	
Electron exposure (e <sup>-</sup> Å <sup>-2</sup> )	60	-	-	60	-
Defocus range (μm)	-1.6~0.6			-2.0~0.8	
Initial particle images (no.)	693,855			253,069	
Software	Relion 3.1	Relion 3.1 & 4β	Relion 4β	Relion 4β	Relion 4β

Final particle images (no.)	662,015	255,000	326,575	325,940	596,902
Map Resolution (Å) FSC threshold	3.1 0.143	3.4 0.143	3.2 0.143	3.4 0.143	3.3 0.143
<b>Refinement</b>					
Initial model used (PDB code)	6YSL	8ZYV	8ZYV	8ZYV	8ZYV
Model resolution (Å) FSC threshold	3.1 0.5	3.4 0.5	3.2 0.5	3.4 0.5	3.3 0.5
Map sharpening B factor (Å <sup>2</sup> )	-133.426	-131.963	-114.517	-135.943	-125.242
<b>Model composition</b>					
Non-hydrogen atoms	8,913	8,910	8,918	8,914	8,713
Protein residues	1,174	1,174	1,174	1,174	1,144
Solvent	5	4	5	3	1
Ligands	2	0	2	0	3
<b>r.m.s. deviations</b>					
Bond lengths (Å)	0.003	0.003	0.004	0.003	0.006
Bond angles (°)	0.488	0.5456	0.529	0.582	0.689
<b>Validation</b>					
MolProbity score	1.4	1.47	1.49	1.51	1.68
Clashscore	7.29	8.67	8.66	9.76	12.44
Poor rotamers (%)	0.21	0.32	0.11	0	0.44
<b>Ramachandran plot statistics (%)</b>					
Preferred	98.4	98.3	97.9	98.1	97.7
Allowed	1.6	1.7	2.1	1.9	2.3
Outlier	0	0	0	0	0

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