

Supplemental Table S1: Cryo-EM data collection, refinement and validation statistics.

	<i>VaPomAB</i>	<i>VaPomAB</i> (MSP1D1 nanodisc)	<i>VaPomAB</i> (Saposin nanodisc)
Data collection and processing			
Microscope	Titan Krios G2		
Voltage (kV)	300		
Magnification (nominal)	96,000x		
Total exposure (e ⁻ /Å ²)	37.98	40.00	37.98
Exposure fractions (no.)	40		
Pixel size (Å)	0.832		
Movies used (no.)	6,444	3,798	1,823
Total picked particles (no.)	3,659,926	959,766	498,542
Final particles (no.)	923,963	66,296	38,438
Box size (pixels)	500	400	400
Symmetry imposed	C1		
Map resolution (Å) (FSC 0.143)	2.48	3.90	6.30
Refinement			
Model composition			
Non-hydrogen atoms	9,926	99,16	
Protein residues	1,312	1,312	
Solvent molecules	12	-	
B-factors (mean; Å ²)			
Protein	75.39	123.52	
Solvent	30.06	-	
R.m.s. deviations			
Bond lengths (Å)	0.006	0.006	
Bond angles (°)	0.874	1.288	
CC (mask)	0.83	0.79	
Refinement resolution (FSC map vs. model (masked)=0.143) (Å)	2.4	3.8	
Validation			
MolProbity score	2.04	2.08	
Poor rotamers (%)	0.00	0.09	
Ramachandran plot			
Favored (%)	97.61	97.23	
Allowed (%)	2.39	2.77	
Disallowed (%)	0.00	0.00	

Supplemental Table S2: bacterial strains

Number	Genotype	Source
n/a	NEB Dh5alpha (cloning strain)	NEB, Ipswich, MA, USA
TH437	Salmonella enterica serovar Typhimurium LT2	J. Roth (University of California, Davis)
EM10372	DmotAB (leaving first and last 15 bp)	Mònica Santiveri et al. 2020
EM12719	LT2 / hhd-pbad33-pomapotb59-e-coli (p46) (CmR)	This study
EM12720	LT2 / hhd-p47-pbad33-pomapotb59-samonella (p47) (CmR)	This study
EM12721	DmotAB / hhd-pbad33-pomapotb59-e-coli (p46) (CmR)	This study
EM12722	DmotAB / hhd-p47-pbad33-pomapotb59-samonella (p47) (CmR)	This study
EM12734	LT2 / pBAD33.1 (Adgene #36267; CmR)	This study
EM12735	DmotAB / pBAD33.1 (Adgene #36267; CmR)	This study
EM13301	DmotAB / pEM13101 (pBAD33.1-pomA(S25C)pomB, Samonella PG-binding domain, CmR)	This study
EM13302	DmotAB / pEM13102 (pBAD33.1-pomA(D31N)pomB, Samonella PG-binding domain, CmR)	This study
EM13303	DmotAB / pEM13103 (pBAD33.1-pomA(R88A)pomB, Samonella PG-binding domain, CmR)	This study
EM13304	DmotAB / pEM13104 (pBAD33.1-pomA(K89A)pomB, Samonella PG-binding domain, CmR)	This study
EM13305	DmotAB / pEM13105 (pBAD33.1-pomA(E96A)pomB, Samonella PG-binding domain, CmR)	This study
EM13306	DmotAB / pEM13106 (pBAD33.1-pomA(E97A)pomB, Samonella PG-binding domain, CmR)	This study
EM13307	DmotAB / pEM13107 (pBAD33.1-pomA(D114R)pomB, Samonella PG-binding domain, CmR)	This study
EM13308	DmotAB / pEM13108 (pBAD33.1-pomA(D114A)pomB, Samonella PG-binding domain CmR)	This study
EM13309	DmotAB / pEM13109 (pBAD33.1-pomA(P151A)pomB, Samonella PG-binding domain, CmR)	This study
EM13310	DmotAB / pEM13110 (pBAD33.1-pomA(M155A)pomB, Samonella PG-binding domain, CmR)	This study
EM13311	DmotAB / pEM13111 (pBAD33.1-pomA(M155L)pomB, Samonella PG-binding domain, CmR)	This study
EM13312	DmotAB / pEM13112 (pBAD33.1-pomA(T158A)pomB, Samonella PG-binding domain, CmR)	This study
EM13313	DmotAB / pEM13113 (pBAD33.1-pomA(V160M)pomB, Samonella PG-binding domain, CmR)	This study
EM13314	DmotAB / pEM13114 (pBAD33.1-pomA(M165A)pomB, Samonella PG-binding domain, CmR)	This study
EM13315	DmotAB / pEM13115 (pBAD33.1-pomA(M169A)pomB, Samonella PG-binding domain, CmR)	This study
EM13316	DmotAB / pEM13116 (pBAD33.1-pomA(I175A)pomB, Samonella PG-binding domain, CmR)	This study
EM13317	DmotAB / pEM13117 (pBAD33.1-pomA(P177A)pomB, Samonella PG-binding domain, CmR)	This study
EM13318	DmotAB / pEM13118 (pBAD33.1-pomA(P177Q)pomB, Samonella PG-binding domain, CmR)	This study
EM13319	DmotAB / pEM13119 (pBAD33.1-pomA(M179A)pomB, Samonella PG-binding domain, CmR)	This study
EM13320	DmotAB / pEM13120 (pBAD33.1-pomA(M179I)pomB, Samonella PG-binding domain, CmR)	This study
EM13321	DmotAB / pEM13121 (pBAD33.1-pomA(L183A)pomB, Samonella PG-binding domain, CmR)	This study
EM13322	DmotAB / pEM13122 (pBAD33.1-pomA(L183M)pomB, Samonella PG-binding domain, CmR)	This study
EM13323	DmotAB / pEM13123 (pBAD33.1-pomA(L183F)pomB, Samonella PG-binding domain, CmR)	This study
EM13324	DmotAB / pEM13124 (pBAD33.1-pomA(L184V)pomB, Samonella PG-binding domain, CmR)	This study
EM13325	DmotAB / pEM13125 (pBAD33.1-pomA(T186A)pomB, Samonella PG-binding domain, CmR)	This study
EM13326	DmotAB / pEM13126 (pBAD33.1-pomA(A190I)pomB, Samonella PG-binding domain, CmR)	This study
EM13327	DmotAB / pEM13127 (pBAD33.1-pomA(M195G)pomB, Samonella PG-binding domain, CmR)	This study
EM13328	DmotAB / pEM13128 (pBAD33.1-pomA(V196F)pomB, Samonella PG-binding domain, CmR)	This study
EM13329	DmotAB / pEM13129 (pBAD33.1-pomA(R232A)pomB, Samonella PG-binding domain, CmR)	This study
EM13330	DmotAB / pEM13130 (pBAD33.1-pomApomB(G20V), Samonella PG-binding domain, CmR)	This study
EM13331	DmotAB / pEM13131 (pBAD33.1-pomApomB(G20W), Samonella PG-binding domain, CmR)	This study
EM13332	DmotAB / pEM13132 (pBAD33.1-pomApomB(T21A), Samonella PG-binding domain, CmR)	This study
EM13333	DmotAB / pEM13133 (pBAD33.1-pomApomB(F22Y), Samonella PG-binding domain, CmR)	This study

EM13334	DmotAB / pEM13134 (pBAD33.1-pomApomB(D24A), Samonella PG-binding domain, CmR)	This study
EM13335	DmotAB / pEM13135 (pBAD33.1-pomApomB(L25F), Samonella PG-binding domain, CmR)	This study
EM13336	DmotAB / pEM13136 (pBAD33.1-pomApomB(S27A), Samonella PG-binding domain, CmR)	This study
EM13337	DmotAB / pEM13137 (pBAD33.1-pomApomB(L28Q), Samonella PG-binding domain, CmR)	This study
EM13338	DmotAB / pEM13138 (pBAD33.1-pomApomB(F32A), Samonella PG-binding domain, CmR)	This study
EM13339	DmotAB / pEM13139 (pBAD33.1-pomApomB(L35V), Samonella PG-binding domain, CmR)	This study
EM13340	DmotAB / pEM13140 (pBAD33.1-pomApomB(L36A), Samonella PG-binding domain, CmR)	This study
EM13341	DmotAB / pEM13141 (pBAD33.1-pomApomB(L36Q), Samonella PG-binding domain, CmR)	This study
EM13783	DmotAB / pEM13778 (pBAD33.1-pomA(K60A)pomB, Samonella PG-binding domain CmR)	This study
EM13784	DmotAB / pEM13779 (pBAD33.1-pomA(T185A)pomB, Samonella PG-binding domain CmR)	This study
EM13785	DmotAB / pEM13780 (pBAD33.1-pomA(L249A)pomB, Samonella PG-binding domain CmR)	This study
EM13786	DmotAB / pEM13781 (pBAD33.1-pomA(D252A)pomB, Samonella PG-binding domain CmR)	This study
EM13787	DmotAB / pEM13782 (pBAD33.1-pomA(ΔAA245-253)pomB, Samonella PG-binding domain CmR)	This study

Supplemental Table S3: Primers

Number	Name	Sequence 5'-3'
5593	Va-PomAS25C-fwd	GCTTGGTGGCtGCATCGGCATGTTTGTCT
5594	Va-PomAS25C-rv	GACAAACATGCCGATGCaGCCACCAAGC
5595	Va-PomAD31N-fwd	CATGTTTGTCaATGTACAGTCGATCC
5596	Va-PomAD31N-rv	GGATCGACGTGACATtGACAAACATG
5597	Va-PomAR88A-fwd	GATGCGGCGgccAAAGGTGGTTTTCTTG
5598	Va-PomAR88A-rv	CAAGAAAACCACCTTTggcCGCCGCATC
5599	Va-PomAK89A-fwd	GATGCGGCGCGTgCGGTGGTTTTTC
5600	Va-PomAK89A-rv	GAAAACCACCgcACGCGCCGCATC
5601	Va-PomAE96A-fwd	CTTGCTCTTGcgGAGATGGAAATAAAC
5602	Va-PomAE96A-rv	GTTTATTTCATCTCcgCAAGAGCAAG
5603	Va-PomAE97A-fwd	CTTGCTCTGAAGccATGGAAATAAAC
5604	Va-PomAE97A-rv	GTTTATTTCATggCTTCAAGAGCAAG
5605	Va-PomAD114R-fwd	GATCTACTGGTTcgCGCCATGATGC
5606	Va-PomAD114R-rv	GCATCATGGCCcgAACCAGTAGATC
5607	Va-PomAD114A-fwd	GATCTACTGGTTGcgGGCCATGATG
5608	Va-PomAD114A-rv	CATCATGGCCcgCAACCAGTAGATC
5609	Va-PomAP151A-fwd	GGCGACGTTGCTgCcGCGATGGGAATG
5610	Va-PomAP151A-rv	CATTCCCATCGCgGcAGCAACGTCGCC
5611	Va-PomAM155A-fwd	CTGCGATGGGAgcGATTGGCACCTTG
5612	Va-PomAM155A-rv	CAAGGTGCCAATCgcTCCCATCGCAG
5613	Va-PomAM155L-fwd	CTGCGATGGGAeTGATTGGCACCTTG
5614	Va-PomAM155L-rv	CAAGGTGCCAATCAgTCCCATCGCAG
5615	Va-PomAT158A-fwd	GGGAATGATTGGCgCgTTGGTTGGTC
5616	Va-PomAT158A-rv	GACCAACCAAcGcGCCAATCATTTCCC
5617	Va-PomAV160M-fwd	GATTGGCACCTTGaTgGGTCTTGTG
5618	Va-PomAV160M-rv	CAACAAGACCcAtCAAGGTGCCAATC
5619	Va-PomAM165A-fwd	GGTCTTGTGCGgcGCTTTCAAACATG
5620	Va-PomAM165A-rv	CATGTTTGAAAGCgcCGCAACAAGACC
5621	Va-PomAM169A-fwd	GCTTTCAAACgcGGATGACCCTAAAGC
5622	Va-PomAM169A-rv	GCTTTAGGGTCATCCgcGTTTGAAAGC
5623	Va-PomAII175A-fwd	GGATGACCCTAAAGCGgcGGACCAGC
5624	Va-PomAII175A-rv	GCTGGTCCcgcCGCTTTAGGGTCATCC
5625	Va-PomAP177A-fwd	CTAAAGCGATTGGAgCAGCAATGGCCG
5626	Va-PomAP177A-rv	CGGCCATTGCTGcTCCAATCGCTTTAG
5627	Va-PomAP177Q-fwd	CTAAAGCGATTGGACaAGCAATGGCCG
5628	Va-PomAP177Q-rv	CGGCCATTGCTtGTCCAATCGCTTTAG
5629	Va-PomAM179A-fwd	GATTGGACCAGCAgcGGCCGTGCAC
5630	Va-PomAM179A-rv	GTGCAACGGCCgcTGCTGGTCCAATC
5631	Va-PomAM179I-fwd	GATTGGACCAGCAATtGCCGTTGCAC
5632	Va-PomAM179I-rv	GTGCAACGGCaATTGCTGGTCCAATC
5633	Va-PomAL183A-fwd	GGCCGTTGCAgcCTTGACCACATTG
5634	Va-PomAL183A-rv	CAATGTGGTCAAGgcTGCAACGGCC
5635	Va-PomAL183M-fwd	GGCCGTTGCAaTgTTGACCACATTG

5636	Va-PomAL183M-rv	CAATGTGGTCAAcAtTGCAACGGCC
5637	Va-PomAL183F-fwd	GGCCGTTGCAiTtTTGACCACATTGTATG
5638	Va-PomAL183F-rv	CATACAATGTGGTCAAAaAaTGCAACGGCC
5639	Va-PomAL184V-fwd	GGCCGTTGCACTCgTGACCACATTG
5640	Va-PomAL184V-rv	CAATGTGGTCAcGAGTGCAACGGCC
5641	Va-PomAT186A-fwd	GCACTCTTGACCgCATTGTATGGCG
5642	Va-PomAT186A-rv	CGCCATACAATGcGGTCAAGAGTGC
5643	Va-PomAA190I-fwd	CACATTGTATGGCattATCCTGTCC
5644	Va-PomAA190I-rv	GGACAGGATaatGCCATACAATGTG
5645	Va-PomAM195G-fwd	GATCCTGTCCAATggGGTGTTTTTCCC
5646	Va-PomAM195G-rv	GGGAAAAACACCccATTGGACAGGATC
5647	Va-PomAV196F-fwd	CCAATATGtTcTTTTCCCTATTGCGG
5648	Va-PomAV196F-rv	CCGCAATAGGGAAAAAgAaCATATTGG
5649	Va-PomAR232A-fwd	GCCAAAACCCGgcAGTGATCGATAG
5650	Va-PomAR232A-rv	CTATCGATCACTgcCGGGTTTTGGC
5651	Va-PotBG20V-fwd	CCGTTATGGATGGtGACATTTCGAG
5652	Va-PotBG20V-rv	CTGCGAATGTCaCCATCCATAACGG
5653	Va-PotBG20W-fwd	CCGTTATGGATGtGGACATTTCGAG
5654	Va-PotBG20W-rv	CTGCGAATGTCCaCATCCATAACGG
5655	Va-PotBT21A-fwd	GGATGGGGgCATTCGCAGATTTGATG
5656	Va-PotBT21A-rv	CATCAAATCTGCGAATGcCCCCATCC
5657	Va-PotBF22Y-fwd	GATGGGGACATaCGCAGATTTGATG
5658	Va-PotBF22Y-rv	CATCAAATCTGCGtATGTCCCCATC
5659	Va-PotBD24A-fwd	GATGGGGACATTTCGCAGcTTTGATGTC
5660	Va-PotBD24A-rv	GACATCAAAgCTGCGAATGTCCCCATC
5661	Va-PotBL25F-fwd	GGACATTCGCAGATTTtATGTCGCTGC
5662	Va-PotBL25F-rv	GCAGCGACATaAAATCTGCGAATGTCC
5663	Va-PotBS27A-fwd	CGCAGATTTGATGgCGCTGCTGATGTG
5664	Va-PotBS27A-rv	CACATCAGCAGCGcCATCAAATCTGCG
5665	Va-PotBL28Q-fwd	GATTTGATGTGCGCaGCTGATGTGTTTC
5666	Va-PotBL28Q-rv	GAAACACATCAGCtGCGACATCAAATC
5667	Va-PotBF32A-fwd	CTGCTGATGTGTgcCTTTGTCTTCTG
5668	Va-PotBF32A-rv	CAGAAGAACAAAGgcACACATCAGCAG
5669	Va-PotBL35V-fwd	GTGTTTCTTTGTTgTTCTGCTCTCG
5670	Va-PotBL35V-rv	CGAGAGCAGAAcAACAAAGAAACAC
5671	Va-PotBL36A-fwd	CTTTGTTCTTgcGCTCTCGTTTTCTG
5672	Va-PotBL36A-rv	CAGAAAACGAGAGCgcAAGAACAAAG
5673	Va-PotBL36Q-fwd	CTTTGTTCTTCaGCTCTCGTTTTCTG
5674	Va-PotBL36Q-rv	CAGAAAACGAGAGCtGAAGAACAAAG
5756	Va-PomAS25C-fwd_rth	tGCATCGGCATGTTTGTCTGATG
5757	Va-PomAS25C-rv_rth	GCCACCAAGCACCATCGCC
5758	Va-PomAR88A-fwd:rth	gccAAAGGTGGTTTTCTTGCTC
5759	Va-PomAR88A-rv_rth	CGCCGCATCGGCCATTTC
5760	Va-PomAK89A-fwd_rth	gcgGGTGGTTTTCTTGCTCTTG
5761	Va-PomAK89A-rv_rth	ACGCGCCGCATCGGCCATTTC
5762	Va-PomAE96A-fwd_rth	cgGAGATGGAAATAAACAAACAC

5763	Va-PomAE96A-rv_rth	CAAGAGCAAGAAAACCACC
5764	Va-PomAM155A-fwd	gcGATTGGCACCTTGGTTGGTC
5765	Va-PomAM155A-rv	TCCCATCGCAGGAGCAACG
5766	Va-PotBF22Y-fwd_rth	aCGCAGATTGTGATGTCGCTGC
5767	Va-PotBF22Y-rv_rth	ATGTCCCCATCCATAACGG
6299	Va-PomA_K60A-fwd	GGTGCGACAgccATTGCTGGCAAAGCC
6300	Va-PomA_T185A -fwd	CGTTGCACTCTTGgCCACATTGTATGGC
6301	Va-PomA_L249A-fwd	CGTGCCgcTGAGATTGACGAGTAACTTG
6302	Va-PomA_D252A-fwd	CTTGAGATTGcCGAGTAACTTGGAGAG
6303	Va-PomA_DeltaC -fwd_rth	TAACTTGGAGAGTCGTGATG
6304	Va-PomA_K60A-rv	GGCTTTGCCAGCAATggcTGTCGCACC
6305	Va-PomA_T185A -rv	GCCATACAATGTGGcCAAGAGTGCAACG
6306	Va-PomA_L249A-rv	CAAGTTACTCGTCAATCTCAgcGGCACG
6307	Va-PomA_D252A-rv	CTCTCCAAGTTACTCGgCAATCTCAAG
6308	Va-PomA_DeltaC -rv_rth	TTCATTGAGGTAGTTCTTCAAG
6299	Va-PomA_K60A-fwd	GGTGCGACAgccATTGCTGGCAAAGCC
6300	Va-PomA_T185A -fwd	CGTTGCACTCTTGgCCACATTGTATGGC
6301	Va-PomA_L249A-fwd	CGTGCCgcTGAGATTGACGAGTAACTTG
6302	Va-PomA_D252A-fwd	CTTGAGATTGcCGAGTAACTTGGAGAG
6303	Va-PomA_DeltaC -fwd_rth	TAACTTGGAGAGTCGTGATG
6304	Va-PomA_K60A-rv	GGCTTTGCCAGCAATggcTGTCGCACC
6305	Va-PomA_T185A -rv	GCCATACAATGTGGcCAAGAGTGCAACG
6306	Va-PomA_L249A-rv	CAAGTTACTCGTCAATCTCAgcGGCACG
6307	Va-PomA_D252A-rv	CTCTCCAAGTTACTCGgCAATCTCAAG
6308	Va-PomA_DeltaC -rv_rth	TTCATTGAGGTAGTTCTTCAAG
6540	p43_fwd	ATGATTGGCACCTTGGTTGG
6541	p43_rev	ATAATGACGAATGCAAAACC
6542	gBlock_p43_fwd	ggttttgcattegtcattatGGCGATGGTGTCTGGTGG
6543	gBlock_p43_rev	ccaaccaagggtgccaatcatTCCCATCGCAGGAGCAAC
6544	p47_fwd	TCGCAGATTGTGATGTCGCTG
6545	p47_rev	GCCATACAATGTGGTCAAGA
6546	gBlock_p47_fwd	tcttgaccacattgtatggcGCGATCCTGTCCAATATG
6547	gBlock_p47_rev	cagcgacatcaaatctggaATGTCCCCATCCATAACG
Sequencing primers		
3117	5'_pBAD24_seq_fw	cgggaccaagccatgacaa
440	3'-pTrc-seq-rv	ggcaaatctgtttatcagac
5497	pomA_Va_check-fwd	GCCTTCATGTTTAAAGCGGA
5498	pomB_Va_check-fwd	GTGTTTCTTTGTTCTTCTGCG
gBlocks		

128	p43_gBlock	GGCGATGGTGCTTGGTGGCAGCATCGGCATGTTTGTGCGATGTCACGT CGATCCTTATTGTCGTTGGTGGCTCAATATTCGTCGTGTTGATGAAGT TCACAATGGGACAGTTTTTTGGTGCGACAgccATTGCTGGCAAAGCCT TCATGTTTAAAGCGGATGAACCCGAAGACCTGATCGCAAAAATTGTG GAAATGGCCGATGCGGCGCGTAAAGGTGGTTTTCTTGCTCTTGAAGAG ATGGAAATAAACAACACATTTCATGCAGAAAGGCATTGATCTACTGGTT GATGGCCATGATGCCGACGTTGTGAGAGCGGCACTCAAAAAAGACATC GCGCTTACGGATGAACGACATACGCAAGGTACTGGTGTATTCGCGCCT TTGGCGACGTTGCTCCTGCGATGGGA
129	p47_gBlock	GCGATCCTGTCCAATATGGTGTTTTTCCCTATTGCGGATAAACTTTCTC TTCGCCGTGACCAAGAAACGCTAAATCGCCGTTTGATCATGGATGGCG TATTAGCGATTCAAGATGGCCAAAACCCGCGAGTGATCGATAGTTACT TGAAGAACTACCTCAATGAATAACTTGGAGAGTCGTGATGGATGATGA AGATAACAAATGCGATTGTCCGCCACCTGGCCTCCCGTTATGGATGGG GACAT