# Science Advances

## Supplementary Materials for

# Fluoxetine targets an allosteric site in the enterovirus 2C AAA+ ATPase and stabilizes a ring-shaped hexameric complex

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Figure S1. (S)-Fluoxetine binds to the nonstructural protein 2C *in vitro*. The binding of SFX to CVB3  $\Delta$ 116-2C was assessed by thermal shift assay. The binding of SFX to  $\Delta$ 116-2C is represented by an increase in melting temperature, which indicates the thermal stabilization of the protein.



Figure S2. Annotated sequence and structure of CVB3  $\Delta$ 116-2C. The fold of CVB3 2C in complex with S-fluoxetine (shown in pink color) is constituted by a Rossman fold core domain prolonged by a sub-4-Cys zinc finger domain and a C-terminal long helix. The  $\beta$ -sheet and  $\alpha$ -helices respectively in green and red color are named accordingly to their position in the structure as depicted in the upper panel of the figure.

EV-A71	1 10	20	30	40	50	
EV-A71 EV71 CV-B3 EV-D68 PV-1 HRV-A2 HRV-A2 HRV-B14 EMCV Aichi-Virus Hepatitis	ASWLKKFNDMAN ASWLKKFNDAAS NNSWLKKFTEAC GDSWLKKFTEAC NDCWFRKFNDAC SDSWLKKFTEAC .SPLKQVNDIF GLKDFNDGAI SF <u>SNWLRDICSG</u> IT	IAAKGLEWIFNKIS AAKGLEWISNKIS IACKGMEWIAVKIC IACKGLEWIAVKIC IAAKGLEWIANKIS IAAKGLEWIANKIS IAARGLEWIGNKIS JAKNLDWAVKTVF JAMRNVEWIGETAF	SKFIDWLKEK SKFIDWLKEK OKFIDWLKEK KFINWLKTK SKFIDWLKEK SKLIEWIKS SKLIEWIKS SKLIEWIKS SKLIEWIKS SKLIEWIKS SKLIEWIKS SKLIEWIKS SKUDWFGTW SKWAHRLLDW STKLKDFYEVI	I I PAAKE KVE I I PAAKE KVE I L PEARE KHE I L PEARE KHE I I PQARE KHE I L PQARE KLE I L PQAKE KLE I RGKAKTDPQ VYGKKKDI L N	FLNNLKQLPLLEN FLNNLKQLPLLEN FUNRLKQLPLLES FVQKLKQLPVIES FVTKLRQLEMLEN FCSKLKQLDILEN YLNELKKLNLYEK A.KLADVHDEIMI ILKDNQQKIEKAI	Q Q Q Q Q Q Q Q Q R H H E
EV-A71 EV-A71 EV71 EV-D68 FV-D68 FV-1 HRV-A2 HRV-B14 EMCV Aichi-Virus Hepatitis	60         70           VSN         LEQSAASQE           ISN         LEQSAASQE           ISN         LEQSAASQE           INT         LEQSAASQE           INT         LEQSASS           INT         LEQSASS           INT         LEQSASS           INT         LEQSASS           INT         LEQSASS           IST         HUSCPSSE           ITT         MHISNPTOF           VES         LRNG           YSDSILALGSEKLF           EADEFCILOIQUE	BO DLEAMFGNVIYL DLEAMFGNVYL DQEQLFSNVQYF XQOALFNNVQYF XQOALFNNVQYF HQEILFNNVWL KREQLFNNVLWLF TQEKIKMEIDTLF AYVECKESFDFF IDHITKSISRCF KFEQYQKGVDLIC	AHFCR HFCR SHYCK SLQSK DLSR DLSR EKLYN KELVSIAQEA KLRTVHSMA	90 KFQPLYATEA (FQPLYATEA (YAPLYALEA (YAPLYALEA (FAPLYAVEA (FAPLYAVES (FLPLYASEA 2AVKEKRTGI (SGPHSSFLN 2VDPNLMVHL)	100 110 KRVYALEKRMNNY KRVYALEKRMNNY KRVFSLEKKMSNY KRVSALERKINNY KRIQKLEHTINNY KRIRELKNKMVNY KRIKTLYIKCDNI AAVCEKFRQKHDH QAIKNYTLAISQH SPLRDCIARVHOK	M M I I I I R R L
EV-A71		 ΤΤ	α1 00000000	000000	β2	
EV-A71 EV-A71 EV-71 CV-B3 EV-D68 PV-1 HRV-B2 HRV-B14 EMCV Aichi-Virus Hepatitis	12 QFKSKHR QFKSKR QFKSKSR QFKSKSR QFKSKR QFKSKR QFKSKR QFKSKR QFKSKR QFKSKR QFKSKR QFKSKR QFKSKR QFKSK QFKSK QFKSK QFKSK QFKSK QFKSK QFKSK QFKSK QFKSK QFKSK	EPVCLIIRGSPG EPVCLIIRGSPG EPVCLIIRGSPG EPVCLIHGSPG EPVCLLVHGSPG EPVCLLVHGPG EPVCVLIHGTPG EPVVIVLRGDAG EPVVVVLYGPPG EPVVVVLYGPG	I 4 9 GKSLATGII GKSVATNLI GKSVATNLI GKSVATNLI GKSVATNLI GKSLTSIV GKSLSSQVI GKSLSSQVI GKSLSSQVI GKSLSSQVI GKSLTSIAL	ARAIADKY ARAIADKY ARAITEKL ARAITEKL GRAIAEHF AKMITN AQAVSKTIFG AQTLSQRLAG ATKICKHYGV	150 RSSVYSLPPDP RSSVYSLPPDP RSSVYSLPPDP RSSVYSLPPDP RSVYSLPPDP RSVYSLPPDP RQSVYSLPPDS RQSVYSLPPDS RQSVYSLPPDS RQSVYSLPPDS RQSVYSLPPDS 	SFX Interaction SFX Interaction AGSINA loop
	ß3	a2	,		64	
<b>EV-A</b> 71	TTT		20000 L90 2	200	TT 210 220	►
EV-A71 EV71 EV-D8 EV-D68 PV-1 HRV-A2 HRV-B14 EMCV Aichi-Virus Hepatitis	HFDGYKOCVVAVM HFDGYKOCVVVM HFDGYKOCVVVM HFDGYKOCVVVM HFDGYKOCVVVM YFDGYDQCEVVIM YFDGYDQCEVVIM FFDGYDQCEVVIM YFDGYHFTU YFDGYLOCVVFFTU YMDGYSCQLVCIT	DICONPOCKOMSI DICONPOCKOMSI DICONPOCKOMSI DICONPOCKOVSI DICONPOCADMIN DINONPOCADMIN DINONPOCADISI DINONPACADMIN DINONPACADMIN DICONPOCSOFT DICONPESSIVAN	FCQMVSTVD FCQMVSTVD FCQMVSTVD FCQMVSTVD FCQMVSTVD FCQMVSVD FCQMVSVD FCQMVSVT FCQMVSTTN FCQMVSTTN FCQMVSAP FQIVSAP	FVPPMASLEE FIPPMASLEE FVPPMASLEE FIPPMASLEE FIPPMASLEE FIPPMASLEP FLPPMASLEE FLPMASLEE HRINMASLEE	KGVSFTSKFVIAS KGVSFTSKFVIAS KGILFTSPFVLAS KGILFTSNFVLAS KGLLFTSNFVLAS KGKAFDSRFVLCS KGKAFDSRFVLCS KGTHFTSQLVVAT KGRHFSSPFIIAT	T T T T T T T T T T T S S
<b>EV-</b> A71	тт тт од	α3 β5	η1 200	α4 2 20000		
EV-A71 EV71 CV-B3 EV-D68 PV-1 HRV-A2 HRV-B14 EMCV Aichi-Virus Hepatitis	Z 3 0 NASNILVPTVSDSI NASNILVPTVSDSI NAGSINAPTVSDSF NSSISPTVAHSD NSNILSPTVAHSD NSNILSPTILNPE NHSLLTPTITSLP NLPEFRPVTIAHYD NFHEPNERAARSMO	ATRRRFYMDCDIE AIRRRFFMDCDIE ALARRFHFDNNIE ALARRFHFDMIE ALARRFAFDMDIC ALVRRFGFDLDIC AMNRRFFLDIDIC AVERRITFDYSVS ALRRVHLRINVT AIDRRLHFKVEVF	EVTDSYKTDL EVTDSYKTEL EVTDSYKTEL EVTDSYKTEL EVTSYKTEL EVTSYKTEL EVTSYKTEL EVTSYKTEL EVTSYKTEL EVTSYKTEL EVTSYKTEL EVTSYKTEL EVTSYKTEL	Z OU RLDAGRAAR RLDAGRAAR SKINMPMSVK /RLDMFKAVE KLNMAMATE SKLMACMSTK SKLNVAAAFR ACYKVLDVER ALNPIPGTQ JDMLNVN	LCTENNTAN.F LCSENNTAN.F ICDDECC.PVN.F LCNPEKCRPTN.Y MC.KNCHQPAN.F C.KDCHQPSN.F PCDVDNRIG.N AFRPTGEAPLPCF SKYFTAQTPLITLF LAKTNDAIK	К К К К К А Q Q D
<i>EV</i> -A71	α5 β6	▶TTT → 2020	α6	2000000000		
: EV-A71 CV-B3 EV-D68 PV-1 HRV-A2 HRV-B14 EMCV Aichi-Virus Hepatitis	280         290           RCSPLVCGKAIQLF           RCSPLVCGKAIQLF           KCCPLVCGKAIQFF           RCCPLVCGKAIQFF           RCCPLVCGKAIQLF           RCCPLVCGKAIQLF           RCCPLVCGKAIQLF           SCVPLVGKAIQLF           NNCLFLEKAGLQFF           SNTVRLDRDSI           NSCVPLINDGH	300 DRKSKVRYSVDTV DRRTQVRYSVDTV DRRTQVRYSUDMI DKSRVRYSVDMI DKSRVRYSIDQI DRTTNIRYSVDQI DRTTNIRYSVDQI DRTKEISLVDV WTPFTNMDEI NVSLMDLLSSI	310 VVSELIREYN VVSELIREYN VVSELIREYN VTEMIKEYN ITTMIINERN KUTAIISDFK VYNIMIEEDR IERAVARIE VDAIVTRIDE VMTVEIRKO	320 WRSATGNTIE IRHSVGTTLE IRHSVGTTLE RNSTQDKLE RRSTQDKLE RRSTQDKE RRSTGNSTQN RKKVLTDSLE RRQVVDVMT RKKVLTTVQ RSTGVSNSLA MTEMELWS	ALFQ ALFQ ALFQ ALFQ TLFQ AIFQ TLVAQ. SLIRRQ 2	

**Figure S3. Multiple sequence alignment of 2C proteins of different picornaviruses.** The sequence alignment of EV-A71 (BrCr), CV-B3 (strain Nancy), PV (strain Sabin), EV-D68 (strain Fermon), HRV-A2, HRV-B14, Encephalomyocarditis virus (EMCV), Aichi virus, and Hepatitis A virus was performed with ClustalOMEGA(*52*). The alignment was subjected to the ESPript 3.0 server(*53*). Conserved residues are highlighted in red with white letters. Highly conserved residues are highlighted in red letters. Secondary structural elements are shown on top of the alignment and are based on the EV-A71 crystal structure (PDB: 5GRB). Sea green Boxes indicate interaction residues with (*S*)-fluoxetine. The orange box indicates the AGSINA loop at the positions 224-229 in which SFX resistance mutations occur.



**Figure S4. Introduction of less stringent mutations in CV-B3 2C cannot rescue CV-B3 replication.** (A) Less stringent mutations were introduced into a recombinant CV-B3 virus encoding a Renilla luciferase reporter gene (Rluc-CV-B3) upstream of the capsid coding region. Infectious RNA was transfected into cells and *Renilla* luciferase was used as a sensitive and quantitative read-out for virus replication.



**Figure S5. Resistance profile of viable 2C mutations of CV-B3.** Viruses with several 2C mutations were tested for their sensitivity against (A) SFX or (B) GuaHCI in a multicycle replication assay. The experimental data displayed represent one out of three independent experiments which were performed in biological triplicates.



Figure S6. Thermal shift analysis of the M175A and P158A mutants. The capacity of SFX to interact with the  $\Delta$ 116-2C mutants was assessed by thermal shift assay. A positive shift of the melting temperature is a sign of thermal stabilization of the protein resulting from an eventual binding with the SFX. A) Representation of M175A and WT (control) melting curves in the absence and presence of SFX. B) Thermal stabilization of WT and P158A by SFX is represented by an increase in the melting temperature (Tm-T0).



**Figure S7. Raising SFX-resistant CVB3 viruses.** CV-B3 viruses resistant to S-fluoxetine (SFX) were raised in a multistep protocol as described previously(*26*). (A) Multicycle viral replication assay was performed to determine SFX sensitivity of CV-B3 viruses resistant to SFX. Therefore, HeLa R19 cells were treated with serial dilutions of SFX and infected with an MOI of 0.001 After 3 days, the cells' viability was determined using an MTS assay. (B) Genotypes of 2C of the raised resistant CV-B3 viruses are shown



**Figure S8. 2C mutations providing resistance to SFX are cross-resistance to dibucaine.** Rluc CV-B3 reporter viruses containing previously identified mutations in the nonstructural protein 2C conferring resistance to several identified 2C inhibitors were used in a single cycle assay. HelaR19 cells were infected with an MOI 0.1 of Rluc-CV-B3 WT, the I227V mutant, the triple mutant A224V-I227V-A229V (designated AVIVAV), the C179Y of C179F mutant and the F190L mutant. One hour after infection, the cells were treated with a serial dilution of dibucaine. The 50% effective concentration EC<sub>50</sub> values displayed are calculated from three independent experiments which were performed in biological triplicates.



Figure S9. Introduction of 224-AGSINA-229 into the infectious clone of EV-A71 results in SFX sensitivity. The 224-AGSINA-229 loop was introduced into the 2C protein of the SFX-insensitive EV-A71 BrCr strain with reverse genetics. The obtained virus was used to determine the SFX-sensitivity in a multicycle assay. In parallel, the cytotoxicity of SFX was determined with a cell viability assay. The experimental data display represents one out of three independent experiments which were performed in biological triplicates.



**Figure S10: Purification of MBP-tagged full-length CV-B2 2C protein.** Size Exclusion Chromatogram for the MBP-tagged full length 2C protein. Elution of the putative hexamer is expected between 14 and 16 mL. The elution volume for molecular weight standards are indicated.



**Figure S11: Overview of the hexΔ116 CV-B3 2C protein expression construct.** Amino acid sequence of the hexΔ116-2C construct showing the positions of the 6xhis tag (red), MBP (green), HRV-3C protease cleavage site (blue), linker (grey), cc-hex-D24 sequence (purple) and residues 116-329 of CV-B3 2C (yellow).



Figure S12. Mass photometry analysis of the monomeric and hexameric 2C constructs. (A) Molecular mass distribution histogram of the MBP tagged monomeric  $\Delta$ 116-2C and (B) hex $\Delta$ 116-2C. The solid lines represent major species that fit with Gaussian functions.



**MBP-Hex-2C-WT** 

**Figure S13. Controls for the ATPase assay.** ATPase activity for the WT hex∆116-2C with or without ATP added, and in the presence of DMSO or a non-2C targeting compound, BF.

![](_page_10_Figure_5.jpeg)

![](_page_11_Figure_0.jpeg)

Figure S14. Assessing the effect of SFX binding site mutations on ATP hydrolysis. ATPase activity for the WT, P158A or M175A hex $\Delta$ 116-2C, with or without SFX added. A representative result of two experiments is shown, each performed in technical triplicates. The data were analyzed by the unpaired, two-tailed Student's t test using GraphPad Prism 8.0. (\*\*\*P < 0.0002, \*\*\*\*P < 0.0001).

![](_page_11_Figure_2.jpeg)

Figure S15: Purification of the hex $\Delta$ 116 CV-B3 2C protein following removal of the MBP tag. A) Coomassie blue-stained SDS-PAGE analysis of the hex $\Delta$ 116-2C construct before and after 3C protease cleavage. B) Size Exclusion chromatogram of the hex $\Delta$ 116-2C after removal of the MBP tag, with the pooled fractions indicated. C) Coomassie blue-stained SDS-PAGE analysis of pooled fractions.

![](_page_12_Figure_0.jpeg)

Figure S16: Cryo-EM processing pipeline for hex $\Delta$ 116 CV-B3 2C incubated with SFX. Single-particle cryo-EM image processing workflow for hex $\Delta$ 116-2C incubated with SFX (see methods for details).

![](_page_13_Figure_0.jpeg)

Figure S17: Estimated resolution for the hex $\Delta$ 116 CV-B3 2C cryo-EM reconstruction. A) Gold-standard Fourier shell correlation (FSC) curve generated from the independent half maps contributing to the ~12 Å resolution density map.

![](_page_14_Figure_0.jpeg)

Figure S18: Crystal packing of  $\Delta$ 116 CV-B3 2C in complex with SFX. A) Crystal packing of the SFX-bound  $\Delta$ 116 CV-B3 2C structures reported here. B) Ribbon diagram of the  $\Delta$ 116 CV-B3 2C structure. The C-terminal a6 and a7 helices, believed to be involved in oligomerization, are colored red. C) Crystal contacts between symmetry related, SFX-bound  $\Delta$ 116 CV-B3 2C molecules showing the affinity of the C-terminal a7 helix towards the Zn binding site region of neighboring molecules within the crystal.

![](_page_15_Figure_0.jpeg)

**Figure S19: Purification of hex\Delta116 CV-B3 2C in the presence or absence of SFX.** Size Exclusion Chromatogram for the MBP-tagged hex116-2C protein in the presence or absence of 30  $\mu$ M SFX. As shown in (A) for the monomeric MBP-tagged 116-2C protein.

 Table S1: Data collection and refinement statistics. Statistics for the highest-resolution

 shell are shown in parentheses

	5S3A	6T3W
Wavelength	0,98	0,98
Resolution range	44.14 - 1.52 (1.574 - 1.52)	39.68 - 1.82 (1.885 - 1.82)
Space group	P 21 21 21	P 21 21 21
Unit cell	48.40 53.18 79.15 90 90 90	47.99 53.00 79.37 90 90 90
Total reflections	178322 (17165)	230223 (17203)
Unique reflections	32092 (3154)	18771 (1846)
Multiplicity	5.6 (5.4)	12.3 (9.3)
Completeness (%)	98.18 (94.87)	99.97 (100.00)
Mean I/sigma(I)	13.69 (1.89)	21.73 (2.16)
Wilson B-factor	16.39	29.35
R-merge	0.07018 (0.8355)	0.06223 (0.8174)
R-meas	0.07759 (0.9264)	0.06496 (0.8656)
R-pim	0.0326 (0.3955)	0.01838 (0.2818)
CC1/2	0.999 (0.646)	1 (0.798)
CC*	1 (0.886)	1 (0.942)
Reflections used in refinement	31556 (2994)	18768 (1846)
Reflections used for R-free	1889 (174)	1878 (185)
R-work	0.1853 (0.2538)	0.1984 (0.2740)
R-free	0.1995 (0.2579)	0.2128 (0.3030)
CC(work)	0.959 (0.800)	0.954 (0.789)
CC(free)	0.952 (0.798)	0.945 (0.730)
Number of non-hydrogen atoms	1873	1739
macromolecules	1623	1620
ligands	24	45
solvent	226	74
Protein residues	210	208

RMS(bonds)	0.007	0.013
RMS(angles)	1.09	1.63
Ramachandran favored (%)	98.36	98.53
Ramachandran allowed (%)	1.64	1.47
Ramachandran outliers (%)	0.00	0.00
Rotamer outliers (%)	0.00	0.00
Clashscore	6.09	5.15
Average B-factor	21.15	36.76
macromolecules	19.69	35.99
ligands	25.48	50.67
solvent	31.11	45.10

#### Table S2. Mutations in SFX coordinating residues do not result in replicating CV-B3.

Several 2C mutations were introduced into an infectious clone of CV-B3. Viral RNA was transcribed *in vitro* and transfected into HeLaR19 or BGM cells. + indicates full CPE observed, +/- indicates partial CPE, - indicates no CPE observed.

	СРЕ																	
	Sequencing result																	
	HeLaR	HeLaR19							BGM									
	Passage 1 Passage 2 Pa			Passa	vassage 3 Pas			assage 1		Passage 2			Passage 3					
Well	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
WT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L157A	+ WT	-	-	+ WT	-	-	+ WT	-	-	-	-	-	-	-	-	-	-	-
P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A	+ P158A
P159A	-	+ P159A A229V	-	-	+ P159A A229V	-	-	+ P159A A229V	+/- P159A A229V	-	-	-	-	-	-	-	-	-
M175A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M175G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M175I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D176A	-	-	-	-	-	-	-	-	-	-	+ WT	-	-	+ WT	-	-	+ WT	-
D176N	-	+ WT	-	-	+ WT	-	-	+ WT	-	-	-	-	-	-	-	-	-	-
L178A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L178I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P182A	-	-	-	-	-	-	-	-	-	-	+ WT	-	-	+ WT	-	-	+ WT	-
D186A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D186N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D186E	-	-	-	-	-	-				-	-	-	-	-	-	-	-	-

 Table S3 Primers list for 2C site directed mutagenesis.
 Changed bases are indicated in lower cases.

2C		5'-3' sequence
mutations		
L157A	FWD	AGTGTACTCAgcaCCGCCAGACC
	REV	GAGCTGTTGAGTTTCTCAG
P158A	FWD	GTACTCACTAgcgCCAGACCCAG
	REV	ACTGAGCTGTTGAGTTTCTCAGC
P159A	FWD	CTCACTACCGgcaGACCCAGATC
	REV	TACACTGAGCTGTTGAGTTTCTC
M175A	FWD	CGTGGTGATTgcgGACGATCTATGC
	REV	GCCTGCTGTTTGTATCCG
M175G	FWD	CGTGGTGATTgggGACGATCTATGC
	REV	GCCTGCTGTTTGTATCCG
M175I	FWD	CGTGGTGATTattGACGATCTATGC
	REV	GCCTGCTGTTTGTATCCG
D176A	FWD	GGTGATTATGgccGATCTATGCC
	REV	ACGGCCTGCTGTTTGTAT
D176N	FWD	GGTGATTATGaacGATCTATGC
	REV	ACGGCCTGCTGTTTGTAT
L178A	FWD	TATGGACGATgcaTGCCAGAATCCTGATG
	REV	ATCACCACGGCCTGCTGT
L178I	FWD	TATGGACGATattTGCCAGAATCCTGATGG
	REV	ATCACCACGGCCTGCTGT
P182A	FWD	ATGCCAGAATgccGATGGGAAAG
	REV	AGATCGTCCATAATCACC
D186A	FWD	TGATGGGAAAgccGTCTCCTTGT
	REV	GGATTCTGGCATAGATCG
D186E	FWD	TGATGGGAAAgagGTCTCCTTGT
	REV	GGATTCTGGCATAGATCG
D186N	FWD	TGATGGGAAAaacGTCTCCTTGT
	REV	GGATTCTGGCATAGATCGTC
A229V	FWD	ATCTATTAATgttCCAACCGTGTCAG
	REV	CCTGCATTGGTCGATGCC

### Table S4: Cryo-EM data collection and image processing

	Hex116-2C + SFX	
Data Collection		
Camera	K2	K3
Datastar mada	Counting	Curren recelution
Detector mode	Counting	Super resolution
Movies collected	1527	6119
Magnification	165 000	64 000

Voltage (kV)	300	300	
Stage tilt (°)	0	30	
Electron exposure (e-/Ų)	50	54	
Defocus range (µm)	1.5-2.5	2-4	
Pixel size (Å)	0.84	0.69	
Image processing			
Pixel size (Å)	1.68	2.76	
Symmetry imposed	C6	C6	
Initial particle images (no.)	41 206	180 069	
Final particle images (no.)	3616	6856	
Map resolution (Å)	N/A	12	
FSC threshold	N/A	0.143	