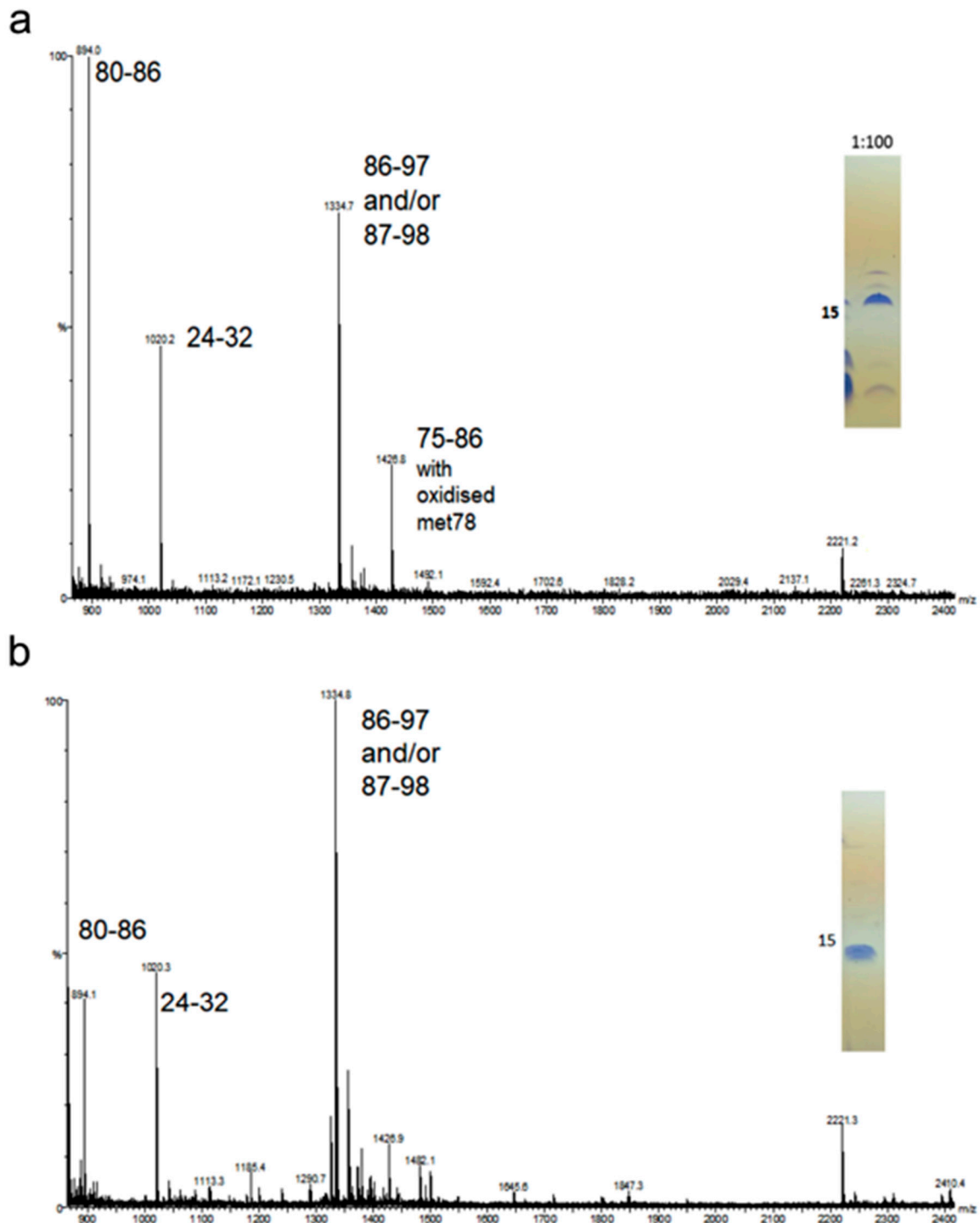
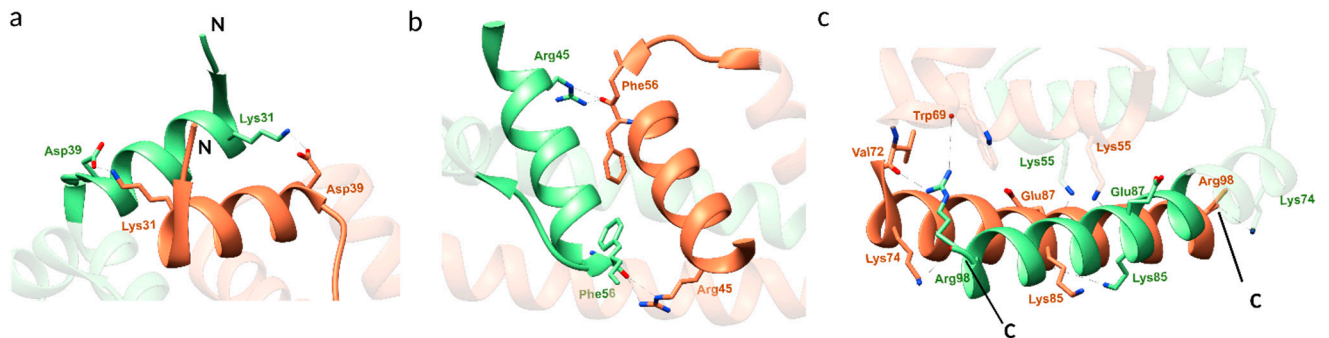


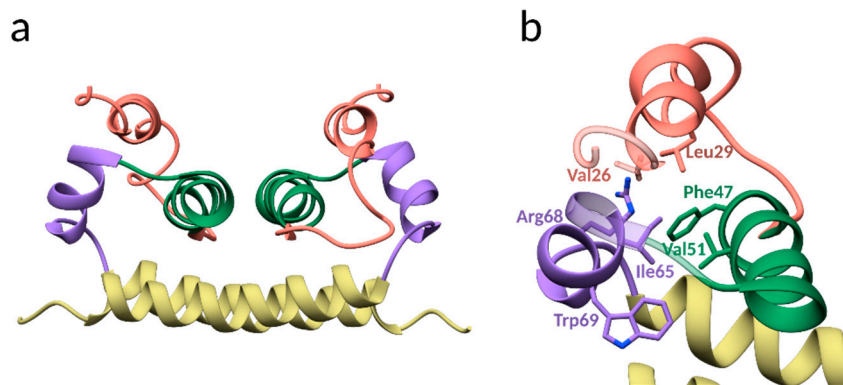
Supplementary Materials



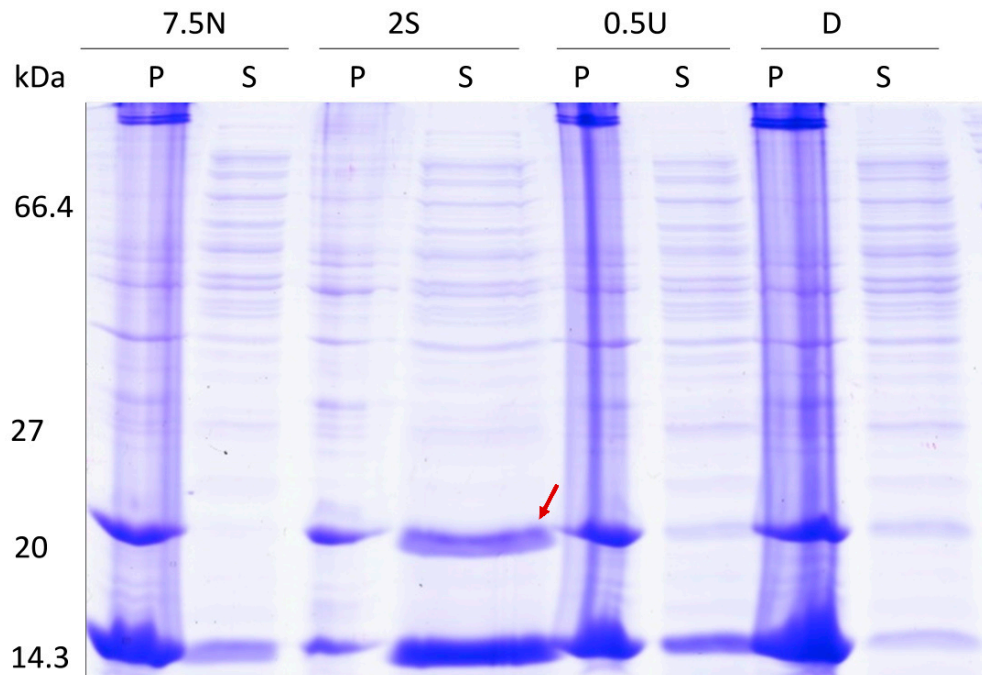
**Figure S1.** Mass-spectrometry analysis of JEV capsid protein. MS analysis of digested (a) and purified capsid (b). SDS-PAGE gels of the samples used for MS analysis are shown. Residue numbers are indicated next to the peaks.



**Figure S2.** Hydrogen bonds at the JEV capsid dimer interface. Hydrogen bonds at the  $\alpha 1$ - $\alpha 1'$ ,  $\alpha 2$ - $\alpha 2'$ , and  $\alpha 3$ - $\alpha 3'$  are shown with dashed lines in figure (a), (b), and (c), respectively. One monomer is in orange. Another is in green.



**Figure S3.** DENV capsid  $\alpha 1$ - $\alpha 3$  bundle. Residues involve in side chain packing of DENV (PDB: 1R6R) capsid  $\alpha 1$ - $\alpha 3$  bundle are labeled. Each monomer coloured by the 1-4  $\alpha$  helices in orange, green, purple, and yellow, respectively.



**Figure S4.** JEV capsid protein lysis buffer screening. Lysis buffer screening demonstrated that capsid protein is soluble in 2 M salt buffer indicating by red arrow and partially soluble in buffer containing urea and detergent. Pellet fractions and suspension fractions were labelled P and S, respectively. Four lysis buffers were the buffer added with the following additives: no additive (7.5N), 2 M NaCl (2S), 0.5 M urea (0.5U), and 0.2% Triton X 100 (D).

**Table S1.** Data collection and refinement statistics.

<b>JEV Capsid</b>	
<b>Data Collection</b>	
Space group	P212121
Cell dimensions	
<i>a, b, c</i> (Å)	46.31, 49.78, 68.25
$\alpha, \beta, \gamma$ (°)	90, 90, 90
Resolution (Å)	38.32-1.98(2.03-1.98)*
<i>R</i> <sub>merge</sub>	0.058(0.862)
<i>R</i> <sub>pim</sub>	0.044(0.686)
<i>I</i> / $\sigma$ <i>I</i>	12.1(1.4)
CC half	0.999(0.551)
Completeness (%)	99.6(99)
Redundancy	4.5(4.3)
<b>Refinement</b>	
Resolution (Å)	38.32-1.98
No. reflections	10871
<i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub>	0.188/0.237
No. atoms	1229
Protein	1144
Water	56
Ethylene glycol	16
Citrate ion	13
<i>B</i> -factors	
Protein	42.77
Water	53.26
Ethylene glycol	52.91
2-propanol	-
Citrate ion	66.22
R.m.s. deviations	
Bond lengths (Å)	0.014
Bond angles (°)	1.428

**Table S2.** Hydrogen bonds between JEV capsid dimer interfacing residues and the distance.

Number	Structure 1	Distance (Å) <sup>1</sup>	Structure 2
1	Lys 31 [NZ]	2.64	Asp 39 [OD1] <sup>2</sup>
2	Arg 45 [NE]	3.11	Phe 56 [O]
3	Arg 45 [NH2]	3.13	Phe 56 [O]
4	Arg 98 [NH2]	3.36	Trp 69 [O]
5	Arg 98 [NH1]	2.94	Val 72 [O]
6	Lys 85 [NZ]	3.87	Lys 85 [O]
7	Lys 55 [NZ]	3.03	Glu 87 [O]
8	Lys 74 [NZ]	2.77	Arg 98 [O]
9	Asp 39 [OD1]	2.69	Lys 31 [NZ]
10	Phe 56 [O]	3.16	Arg 45 [NH2]
11	Phe 56 [O]	3.07	Arg 45 [NE]
12	Glu 87 [O]	3.00	Lys 55 [NZ]
13	Arg 98 [O]	2.98	Lys 74 [NZ]

<sup>1</sup> Assembly analysis in the program PISA [50].

**Table S3.** Salt bridges between JEV capsid dimer interfacing residues.

Number	Structure 1	Distance (Å) <sup>1</sup>	Structure 2
1	Lys 31 [NZ]	2.64	Asp 39 [OD1]
2	Lys 74 [NZ]	2.77	Arg 98 [O]
3	Asp 39 [OD1]	2.69	Lys 31 [NZ]
4	Arg 98 [O]	2.98	Lys 74 [NZ]

<sup>1</sup> Assembly analysis in the program PISA.

**Table S4.** JEV capsid dimer interfacing residues reported with accessible (ASA) and buried surface area (BSA), solvation energy effect ( $\Delta G$ ) and conservation score.

(The values are reported from one monomer)

Number	Residue	ASA ( $\text{\AA}^2$ )	BSA ( $\text{\AA}^2$ )	$\Delta G$ (kcal/mol)	Conservation <sup>1</sup>
1	Leu27	83.65	21.41	0.34	1
2	Val30	117.35	93.60	1.5	2*
3	Lys31	144.46	90.81	0.19	8
4	Val33	69.18	18.08	0.29	3*
5	Val34	64.36	56.65	0.91	6*
6	Met35	100.45	54.43	1.29	1
7	Leu37	10.72	9.71	0.16	5*
8	Leu38	49.69	37.96	0.59	3*
9	Asp39	74.39	20.45	-0.36	2*
10	Arg45	84.22	18.52	-0.08	6*
11	Phe46	93.98	43.04	0.67	2*
12	Ala49	41.95	41.95	0.56	7
13	Leu50	24.80	24.80	0.38	2*
14	Ile51	19.52	7.19	0.12	5*
15	Thr52	28.95	28.56	0.17	5*
16	Phe53	92.37	91.73	1.47	7*
17	Phe54	22.78	16.86	0.27	5*
18	Lys55	164.75	92.62	-1.11	7*
19	Phe56	146.69	144.04	1.49	6*
20	Thr57	60.84	42.49	0.44	6*
21	Leu59	102.50	49.22	0.79	7*
22	Ala60	89.25	18.81	-0.10	5*
23	Thr62	52.30	27.04	0.43	8
24	Trp69	87.57	51.05	0.60	8
25	Lys70	155.76	6.41	0.02	
26	Lys72	25.04	11.51	-0.11	
27	Lys74	158.12	83.67	-0.29	6*
28	Ala77	16.40	16.40	0.26	9
29	Met78	106.53	38.83	0.97	7*
30	Leu81	56.97	56.80	0.77	8
31	Thr82	69.35	21.75	0.35	2*
32	Phe84	36.14	36.14	0.51	7*
33	Lys85	156.49	85.08	-0.30	7
34	Glu87	97.01	47.74	0.13	6*
35	Leu88	79.21	79.04	1.24	7
36	Gly89	30.81	14.57	0.21	5*
37	Thr90	93.35	9.88	0.16	4*
38	Leu91	108.63	90.54	1.44	7
39	Ile92	91.95	74.87	1.19	6*
40	Val95	102.29	87.78	1.23	4*
41	Asn96	114.80	45.24	-0.49	8
42	Lys97	165.54	0.48	0.01	3*
43	Arg98	130.94	29.07	0.03	9

<sup>1</sup> Amino acid conservation scores are given by ConSurf. [51] (9 = conserved and 1 = variable) The following UniProtKB were submitted for conservation score calculation: W0LHC1, A0A1B2CW17, A0A0A7AAT7, W0LLY6, Q32ZE1, P12823, P14335, Q89277, P07720, and P27395.

- Below the confidence cut-off [51].

- **References**

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- 50. Krissinel, E.; Henrick, K. Inference of macromolecular assemblies from crystalline state. *J. Mol. Biol.* **2007**, *372*, 774–797. doi:10.1016/j.jmb.2007.05.022.
- 51. Landau, M.; Mayrose, I.; Rosenberg, Y.; Glaser, F.; Martz, E.; Pupko, T.; Ben-Tal, N. ConSurf 2005: the projection of evolutionary conservation scores of residues on protein structures. *Nucleic Acids Res.* **2005**, *33*, W299-302. doi:10.1093/nar/gki370.