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Cryo-EM structure of an antibody that neutralizes dengue virus type 2 by locking E protein dimers

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Abstract

There are four closely-related dengue virus (DENV) serotypes. Infection with one serotype generates antibodies that may cross-react and enhance infection with other serotypes in a secondary infection. We demonstrated that DENV serotype 2 (DENV2)–specific human monoclonal antibody (HMAb) 2D22 is therapeutic in a mouse model of antibody-enhanced severe dengue disease. We determined the cryo–electron microscopy (cryo-EM) structures of HMAb 2D22 complexed with two different DENV2 strains. HMAb 2D22 binds across viral envelope (E) proteins in the dimeric structure, which probably blocks the E protein reorganization required for virus fusion. HMAb 2D22 “locks” two-thirds of or all dimers on the virus surface, depending on

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The data presented in this paper are tabulated in the main paper and in the supplementary materials. Coordinates of 4°C-2D22-PVP94/07, 37°C-2D22-PVP94/07 and BF-37°C-2D22-NGC class II were deposited in the Protein Data Bank under accession codes 4UIF, 5A1Z, and 4UIH, respectively The cryo-EM maps of 4°C-2D22-PVP94/07, 37°C-2D22-PVP94/07, 4°C-2D22-NGC, BF-37°C-2D22-NGC Class I, BF-37°C-2D22-NGC Class II, AF-37°C-2D22-NGC Class I, and AF-37°C-2D22-NGC Class II were deposited in the Electron Microscopy Database under accession numbers EMD-2967, EMD-2996, EMD-2997, EMD-2999, EMD-2968, EMD-2998, and EMD-2969, respectively.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Supplementary Text

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the strain, but neutralizes these DENV2 strains with equal potency. The epitope defined by HMAb 2D22 is a potential target for vaccines and therapeutics.

Dengue virus (DENV), consisting of four serotypes (DENV1 to 4), is a major human pathogen transmitted by mosquitoes (1, 2). DENV causes disease ranging from mild dengue fever to the severe dengue hemorrhagic fever/dengue shock syndrome. Preexisting antibodies against one serotype can enhance infection by virus of a second serotype. This is probably due to the targeting of virus complexed with non-neutralizing antibodies to monocytic cells via interaction with the Fcγ-receptor, thereby increasing virus infection, a process called antibody-dependent enhancement (ADE) of infection (3). Therefore, a safe dengue vaccine should elicit equivalent levels of potently neutralizing responses against all four serotypes. Recent phase 3 clinical trials of a tetravalent vaccine showed poor efficacy, especially against DENV2 (4, 5).

Previous in vitro studies showed that DENV2-specific human monoclonal antibody (HMAb 2D22) has potent neutralization capacity (6). We showed that HMAb 2D22 protects against DENV2 when the antibody is administered before (Fig. 1A) or after (Fig. 1B) DENV2 inoculation in an AG129 mouse model (supplementary text). This indicates the potential of using this HMAb as both a prophylactic and therapeutic agent. We also showed that therapeutic administration of the LALA mutant variant of HMAb 2D22 (which abolishes Fc receptor binding) to AG129 mice pretreated with polyclonal DENV1 serum and then inoculated with DENV2, prevents development of antibody-enhanced lethal vascular leak disease (supplementary text) (Fig. 1C).

DENV-neutralizing antibodies primarily target the viral envelope (E) protein. The E protein contains three domains: DI, DII, and DIII (fig. S1). The cryo–electron microscopy (cryo-EM) structure of DENV2 at 4°C (7) showed E proteins arranged in icosahedral symmetry, with three individual E proteins (A, B, and C molecules) in each asymmetric unit (fig. S1). The E proteins exist as dimers, and three of the dimers lie parallel to each other, forming a raft (7, 8). The 30 rafts are arranged in a herringbone pattern on the virus surface. This structure represents DENV that was grown in mosquito cells (28°C) and then kept at 4°C. However, when exposed to 37°C, the surface proteins of three DENV2 laboratory-adapted strains (NGC, WHO, and 16681) undergo structural rearrangement (9, 10), resulting in bumpy-surfaced expanded virus particles (fig. S2). This was also observed in a mouse-adapted DENV2 strain (fig. S2). On the other hand, the virus particles of clinical isolate DENV2 PVP94/07 did not undergo structural changes at 37°C (fig. S2). Thus, we solved the cryo-EM structures of Fab 2D22 complexed with both DENV2(PVP94/07) and DENV2(NGC) strains (fig. S2).

The cryo-EM structures of Fab 2D22:DENV2 (PVP94/07) at 4° and 37°C were determined to a resolution of 6.5 Å (Fig. 2A and figs. S3 and S4) and 7 Å (figs. S3 and S5, A and B), respectively. As the maps were very similar, the 4°C-2D22-PVP94/07 structure was used to identify the Fab-E protein interactions. There are 180 copies of the Fabs on the virus (Fig. 2A and table S1). The Fab binds across E proteins within a dimer (Fig. 2, B and C). The interactions of the Fabs with each of the three dimers (A-C′, B-B′, and C-A′) in a raft vary slightly (Fig. 2C and table S2). Some of the Fabs also bind to the E protein inter-dimer and

inter-raft interface. However, these additional residues are unlikely to be important for antibody binding, because the studies described below show that HMAb 2D22 also binds to the expanded 37°C-DENV2(NGC) structure, which has an altered quaternary structure. In addition, increasing the contour of the 4°C-2D22-PVP94/07 cryo-EM density map showed that the three individual Fab molecule densities in an asymmetric unit are equally strong (fig. S6). This suggests that the interaction of a Fab with an E protein dimer, which is common between these Fabs in the asymmetric unit, is sufficient for binding.

The light chain of Fab 2D22 bound to DIII and the glycan loop on DI of one E protein, whereas the heavy chain bound to DII, including the fusion loop, of another (Fig. 2C, fig. S7, and table S2). The Fab also caused the glycan-containing loop on DI on an E protein to change in position (Fig. 2D and fig. S4D). Examination of the possible binding of two arms of an immunoglobulin G (IgG) molecule to the DENV virion surface showed that they could bind to adjacent E protein dimers but not to those farther apart (fig. S8).

Previous studies showed that the DENV2 NGC strain can change in structure at 37°C (fig. S2) (9), suggesting that antibody binding may be affected. We solved the structure of the 2D22-NGC DENV2 complex at 4° and 37°C. The 4°C cryo-EM structure of the NGC-2D22 complex (fig. S9A and table S1) was similar to the structure of the Fab 2D22: DENV2(PVP94/07) complex (Fig. 2A and fig. S5A).

There are two possible scenarios for how this antibody could bind to DENV2 (NGC) when infecting humans: It may bind to the virus before and/or after it has changed in structure at 37°C. Therefore, we examined the virus-Fab complex structures when the antibody was added before (BF-37°C-2D22-NGC) or after (AF-37°C-2D22-NGC) shifting the temperature to 37°C. In both BF-37°C-2D22-NGC and AF-37°C-2D22-NGC samples, two classes of particles (class I and II) were observed. All class I structures were similar to the Fab complexed to the unexpanded virus structure (fig. S9, B and C). Therefore, only class II structures will be described.

The class II BF-37°C-2D22-NGC structure (20 Å resolution, fig. S10) showed 120 Fab molecules on the virus surface (Fig. 3A, fig. S11, and table S1), with all E protein dimers on a higher radius as compared to the unexpanded 4°C control complex structure (Fig. 3B, i). Fab molecules remained bound to all A-C dimers, whereas those on the B-B′ dimer had disassociated (Fig. 3A, right). In the BF-37°C-2D22-NGC structure, the Fab that was previously bound to the B-B′ dimer at 4°C (fig. S9A) must have dissociated when the temperature was increased. Movement of the A-C dimers from their position in the 4°C control structure to that in the BF-37°C-2D22-NGC structure would cause clashes of these E proteins with the Fab that was bound to the B-B′ dimer (Fig. 3C). This steric conflict may “knock off” the Fab and cause the B-B′ dimer to rotate, as observed in the BF-37°C-2D22-NGC structure. Also, as suggested by the uncomplexed stage 3 DENV2(NGC) 37°C structure, the B-B′ dimer may dissociate, contributing to the detachment of Fab as observed in the BF-37°C-2D22-NGC structure.

The class II BF-37°C-2D22-NGC structure may represent a structure trapped at an intermediate stage of expansion at 37°C. Previous cryo-EM studies of the uncomplexed

37°C DENV2(NGC) sample showed four stages of structural change (9), of which only stage 1 and 3 structures were interpreted. The first stage is similar to the unexpanded structure. The third stage showed that all dimers had moved to a higher radius. The A-C dimer rotated, whereas the B-B′ dimer dissociated from each other. Comparison of the class II BF-37°C-2D22-NGC structure with the DENV2 37°C stage 3 structure (Fig. 3B, ii) showed similar organization to that of the A-C dimers. The B-B′ dimer, on the other hand, seemed to be a transitional structure between the DENV2 NGC 37°C stage 1 and 3 structure, as it lay at a radius in between these two structures (Fig. 3B, i and ii).

The ~21 Å resolution class II AF-37°C-2D22-NGC cryo-EM map (Fig. 4, A and B, and fig. S10) showed clear Fab densities near the fivefold vertices and much weaker Fab densities near the threefold vertices. The E protein densities were sparse; therefore, the map was not interpreted. The positions of Fab density near the fivefold vertices was similar to those in the BF-37°C-2D22-NGCmap, but not those near the threefold vertices. The Fab densities near the fivefold and threefold vertices probably represent those that are bound to each end of the A-C′ dimer. Neutralization profiles of BF-37°C-2D22-NGC and AF-37°C-2D22-NGC samples were similar (fig. S12B), suggesting that the poor Fab density near the threefold vertices may be due to local movement in the structure rather than low Fab occupancy.

DENV fusion in endosomes requires E protein dimers to disassociate and then reassociate into trimeric structures. Fab 2D22 locks both ends of all dimers on DENV2(PVP94/07), thereby preventing E protein reorganization. In the BF-37°C-2D22-NGC and AF-37°C-2D22-NGC samples, only two-thirds of the dimers on the virus surface are locked. The remaining free dimer in each raft is probably unable to form trimers. Indeed, HMAb 2D22 effectively neutralized DENV2 strains PVP94/07 and NGC, even though the latter bound one-third fewer antibody molecules (fig. S12A).

Many human antibodies that strongly neutralize dengue bind to quaternary epitopes (epitopes involving more than one E protein molecule) (6, 11, 12). Human antibodies that neutralize DENV serotypes 1 and 3 bound to quaternary epitopes, which require virion assembly (11, 12). In contrast, HMAb 2D22 binds to a simpler epitope that requires only the formation of E homodimers. Several DENV serotype cross-neutralizing human antibodies (13) were also shown to bind E protein dimer epitope (EDE) (fig. S13, B, C, and D). These EDEs are largely similar to 2D22 epitope (fig. S13, A, B and C). Compared to HMAb C8 and C10 (fig. S13, A, B, and C), HMAb 2D22 has more interactions on DIII that are unique to DENV2 (fig. S13E), leading to its serotype specificity.

The DENV2 surface is more dynamic than that of the other serotypes (9, 10). Thus, antibodies that bind across different dimers and rafts may lose potency, depending on the temperature and strain of DENV2. Therefore, antibodies binding to “simpler” epitopes, such as monomers or dimers, may be more effective against this serotype.

In areas of high dengue endemicity, a potential therapeutic needs to be protective in the presence of preexisting antibodies (14). Certain MAb LALA variants protect therapeutically against an ADE infection, because they are neutralizing and at the same time suppress the enhancing potential of preexisting fusion loop antibodies by displacing their binding (15–

17). In contrast, highly neutralizing DIII MAbs that do not block fusion loop enhancing antibodies protect in high-dose-DENV2-lethal, but not ADE-DENV2-lethal challenge (Fig. 1C) (17). The increased efficacy of HMAb 2D22 may be due to its ability to lock E proteins and also block the binding of low-affinity fusion-loop enhancing antibodies.

The molecular features of the 2D22 epitope and the ability of HMAb 2D22-LALA to prevent ADE will aid in the development of vaccines and therapeutics, respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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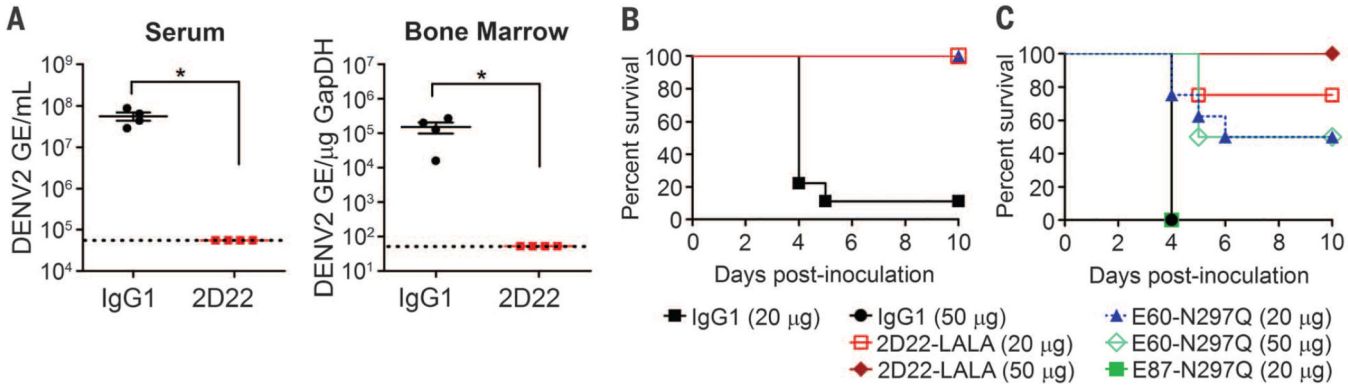

Fig. 1. Prophylactic and therapeutic efficacy of HMAb 2D22 and 2D22-LALA in DENV2-inoculated mice

(**A**) Prophylactic studies. AG129 mice injected with HMAb 2D22 24 hours before sublethal challenge with DENV2 strain D2S10 showed significant reduction in serum viremia and bone marrow viral load, compared to the control mice receiving IgG1 isotype control. The DENV2 limit of detection (LOD) is indicated by the dashed line. Significance was determined using a two-tailed Wilcoxon rank-sum test (*$P < 0.05$). Shown is one of two representative experiments, with $n = 4$ mice per group. (**B**) Therapeutic efficacy after high-dose-DENV2-lethal infection. AG129 mice were first inoculated with DENV2 D2S10, and then 20 μg of HMAb 2D22-LALA was administered 24 hours later. Chimeric human-mouse MAb E60-N297Q or IgG1 isotype controls were used to treat control animals. Shown are the combined results from two independent experiments, with $n = 9$, 8, or 4 mice for the IgG1, 2D22-LALA, or E60-N297Q groups, respectively. Significance was determined using the Mantel-Cox log-rank test ($P < 0.001$, 2D22-LALA versus IgG1). (**C**) Therapeutic efficacy after ADE-DENV2-lethal infection. AG129 mice were administered a lethal enhancing dose of anti-DENV1 serum 24 hours before inoculation with DENV2 D2S10. The indicated MAb was delivered intraperitoneally 24 hours after inoculation. The results from two independent experiments are shown, with $n = 8$ mice for the IgG1, 2D22-LALA (20 or 50 μg) and E60-N297Q (20 μg) groups, $n = 4$ for the group receiving 50 μg of E60-N297Q, and $n = 3$ for E87-N297Q. Mice receiving 20 or 50 μg of 2D22-LALA displayed a significant level of protection compared to IgG1 control mice ($P < 0.001$, Mantel-Cox log-rank test).

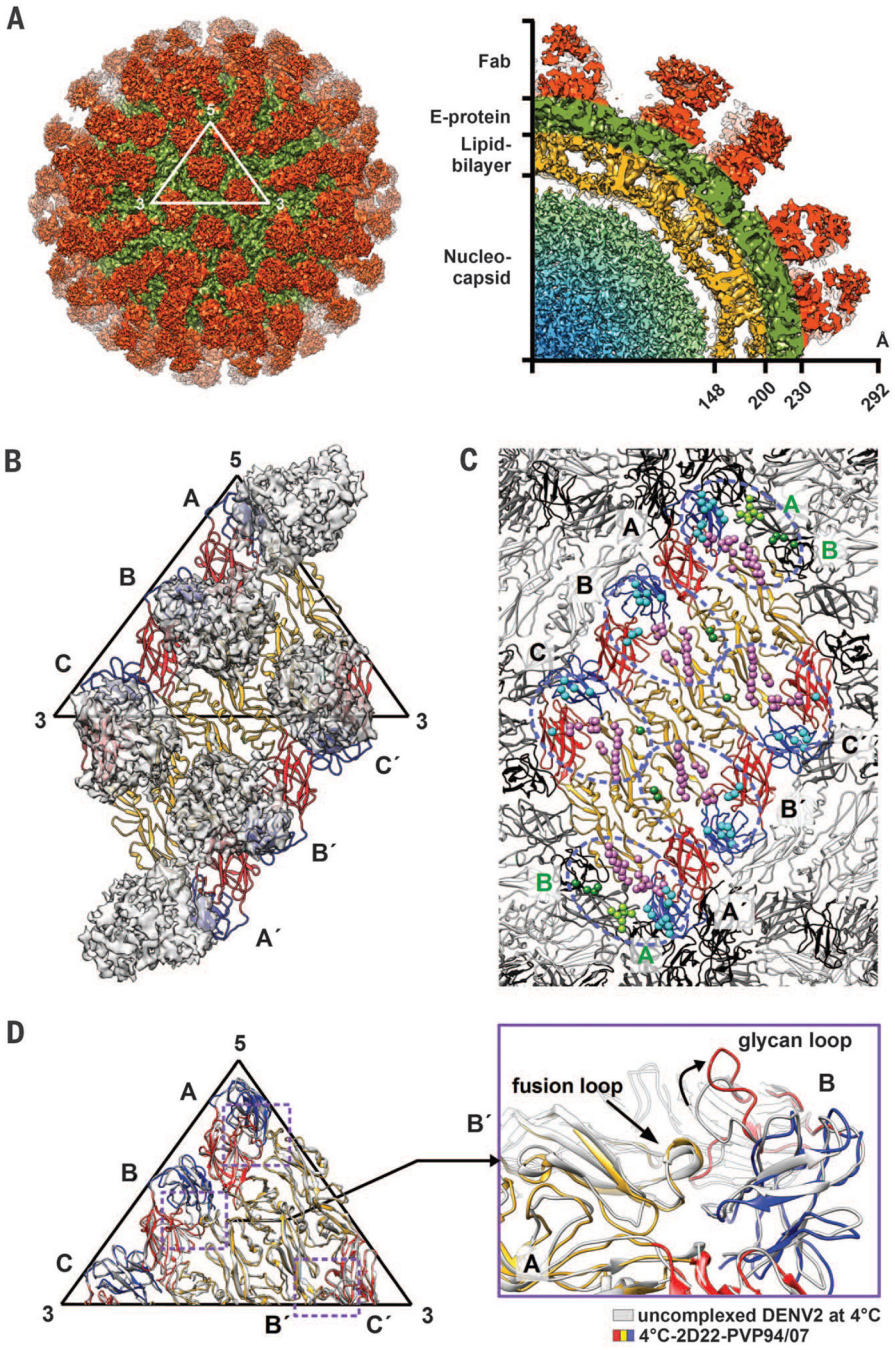

Fig. 2. The 6.5 Å resolution cryo-EM structure of 4° C-2D22-PVP94/07

(**A**) The surface (left) and cross section (right) of the cryo-EM map. One icosahedral asymmetric unit is indicated by a white triangle. (**B**) Three Fab 2D22 molecules bind per asymmetric unit. The three individual E proteins in an asymmetric unit are labeled as molecules A, B, and C. The same molecules from an adjacent asymmetric unit in a raft structure are labeled as A′, B′, and C′. DI, DII, and DIII of E protein are colored in red, yellow, and blue, respectively. (**C**) The 2D22 epitopes on an E protein raft. The epitopes on molecules A, B, and C are largely similar; however, that on molecule A also has some

interactions with adjacent E proteins. The DI, DII, and DIII of the surrounding E proteins are colored in light gray, gray, and black, respectively. The residues that interact with heavy and light chains of the Fab are shown as violet and cyan spheres, respectively. Additional residues from adjacent E protein dimers that formed part of the epitope are shown as green spheres. The boundary of each epitope is indicated with a light blue dotted circle. (**D**) The glycan loop (residues 144 to 157) on one E protein moves away from the fusion loop of the opposite E protein in the dimer when Fab is bound. The E protein of the uncomplexed DENV2 (gray) is superimposed onto the E protein of the complexed structure.

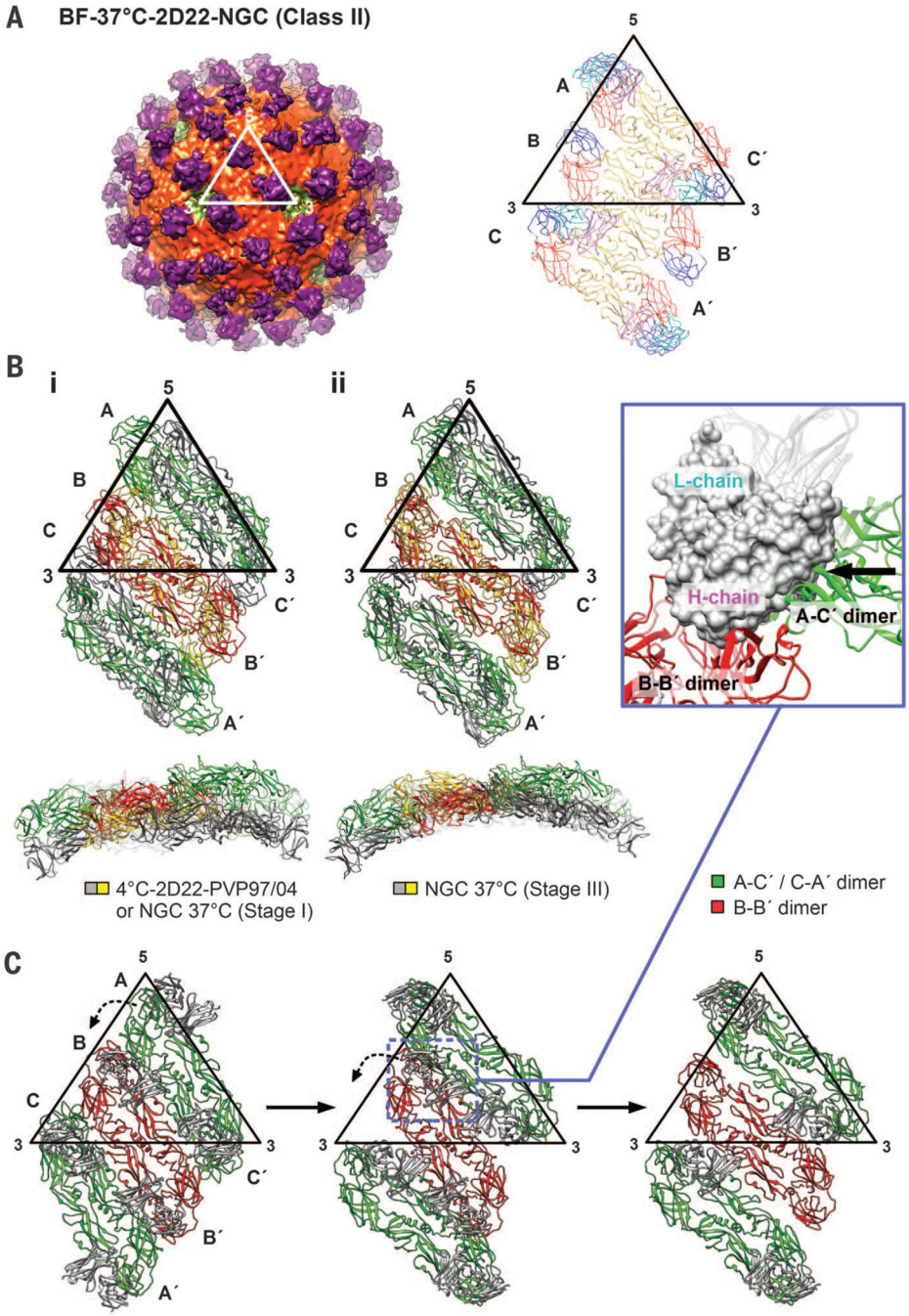

Fig. 3. Cryo-EM structure of BF-37°C-2D22-NGC (Class II)

(**A**) Cryo-EM map of BF-37°C-2D22-NGC (Class II) (left) and the fitted structure of the variable region of the Fab complexed with E dimers on a raft (right). The Fab 2D22 heavy and light chains are colored in violet and cyan, respectively. (**B**) (i) The E proteins of BF-37°C-2D22-NGC are on a higher radius than those of 4°C-2D22-NGC or DENV2(NGC) stage 1. (ii) The E protein arrangement of BF-37°C-2D22-NGC is more similar to that of the 37°CDENV2(NGC) (stage 3) structure (top). The A-C′ dimer of BF-37°C-2D22-NGC is on a slightly higher radius, whereas the B-B′ are located lower when compared to the stage 3

NGC structure. (**C**) Postulated movement of the Fab-E protein complex from the 4°C-2D22-NGC to the BF-37°C-2D22-NGC structures. The partial lifting and rotation of the A-C dimers would knock off the bound Fab on the B-B′ dimer (zoomed-in panel). This may then be followed by the rotation of the B-B′ dimer.

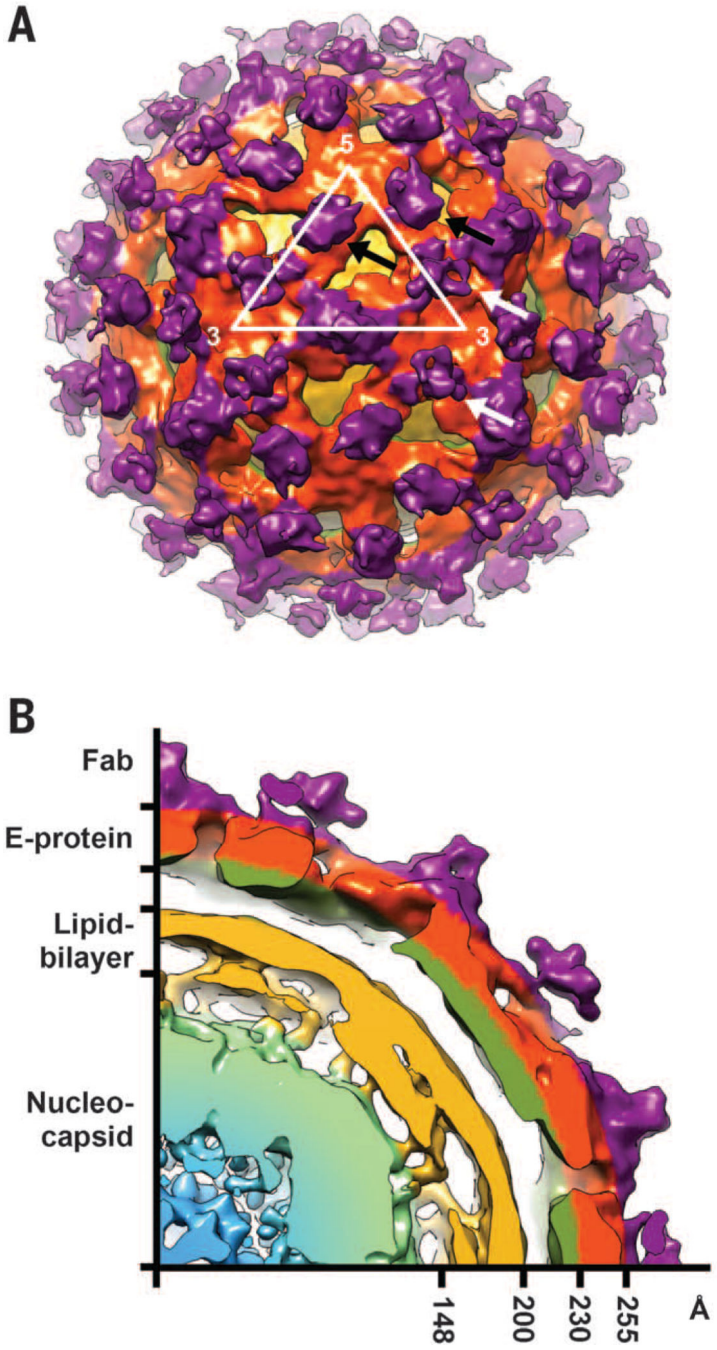

Fig. 4. The cryo-EMmap of AF-37°C-2D22-NGC

(**A**) Surface of the cryo-EM map and (**B**) its central cross section. Fabs bound near the five- and threefold vertices are indicated by black and white arrows, respectively.