Structural determination of Fab-dimerized glycan reactive (FDG) antibodies

In this project we will solve structures of novel FDG antibodies to define their Fab-dimer interface and their epitopes. We have solved several structures of similar complexes (Figures 1 and 2). These provide preliminary data for the feasibility of this project.

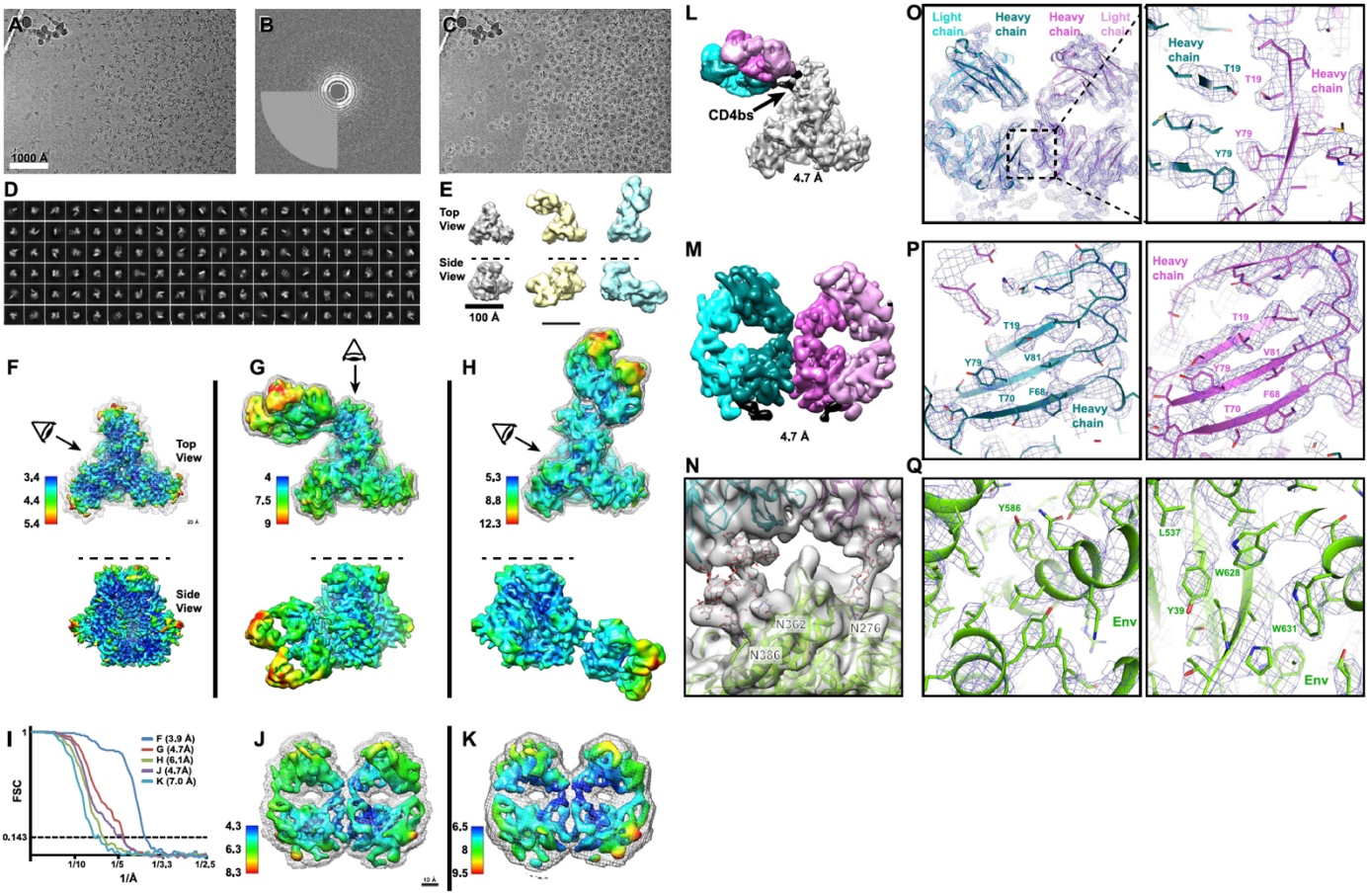

Figure 1. Structural determination of DH898.1 complex with HIV-1 Env. (A) Representative micrograph; (B) Power spectrum of micrograph, and fitted contrast transfer function; (C) Picked particles in white circles; and (D) Representative 2D class averages. (E) Ab initio volumes that showed three particle populations: free trimer, Fab-dimer bound to glycans near the CD4-binding site (bs), and Fab-dimer bound to glycans near the base of the V3 loop (left to right) seen in top and side views (top to bottom). Dashed line in side view indicated viral membrane location. (F) Top and side view of free soluble stabilized recombinant HIV-1 Env trimer (SOSIP) colored by local resolution. Mesh surface indicates mask used for FSC calculation. Eye indicates viewing direction in side view. (G) HIV-1 Env SOSIP with Fab-dimer bound to glycans near the CD4bs. (H) HIV-1 Env SOSIP with Fab-dimer bound to glycans near the base of the V3 loop. (I) Gold standard Fourier Shell Correlation (FSC) curves for F-H and J-K indicating global resolution ranging from 3.9 – 7.0 Å . (J) Local refinement of Fab-dimer from CD4bs particle set. (K) Local refinement of Fab-dimer only from V3-glycan bound particle set. (L) Segmented cryo-EM map showing Fab-dimer bound near the CD4bs. Gold-standard FSC resolution is indicated below each map. (M) Local refinement of the Fab-dimer bound near to the CD4bs of a HIV-1 Env SOSIP. (N) Close-up view of the epitope near the CD4bs with a HIV-1 Env SOSIP and Fab-dimer models shown as ribbons; glycans shown as sticks; and the cryo-EM map shown as a transparent surface. (O) (left) Local refined, density modified map of the DH898.1 Fab dimer with cryo-EM reconstruction shown as blue mesh and underlying fitted model in cartoon representation, and (right) Zoomed-in view of the Fab dimer interface with select interfacial residues shown as sticks. (P) Views of the Fab dimer interface rotated 90□ clockwise (left) or counter-clockwise (right) relate to the view shown in panel O. (Q) Zoomed-in views of Env regions in the apo structure.

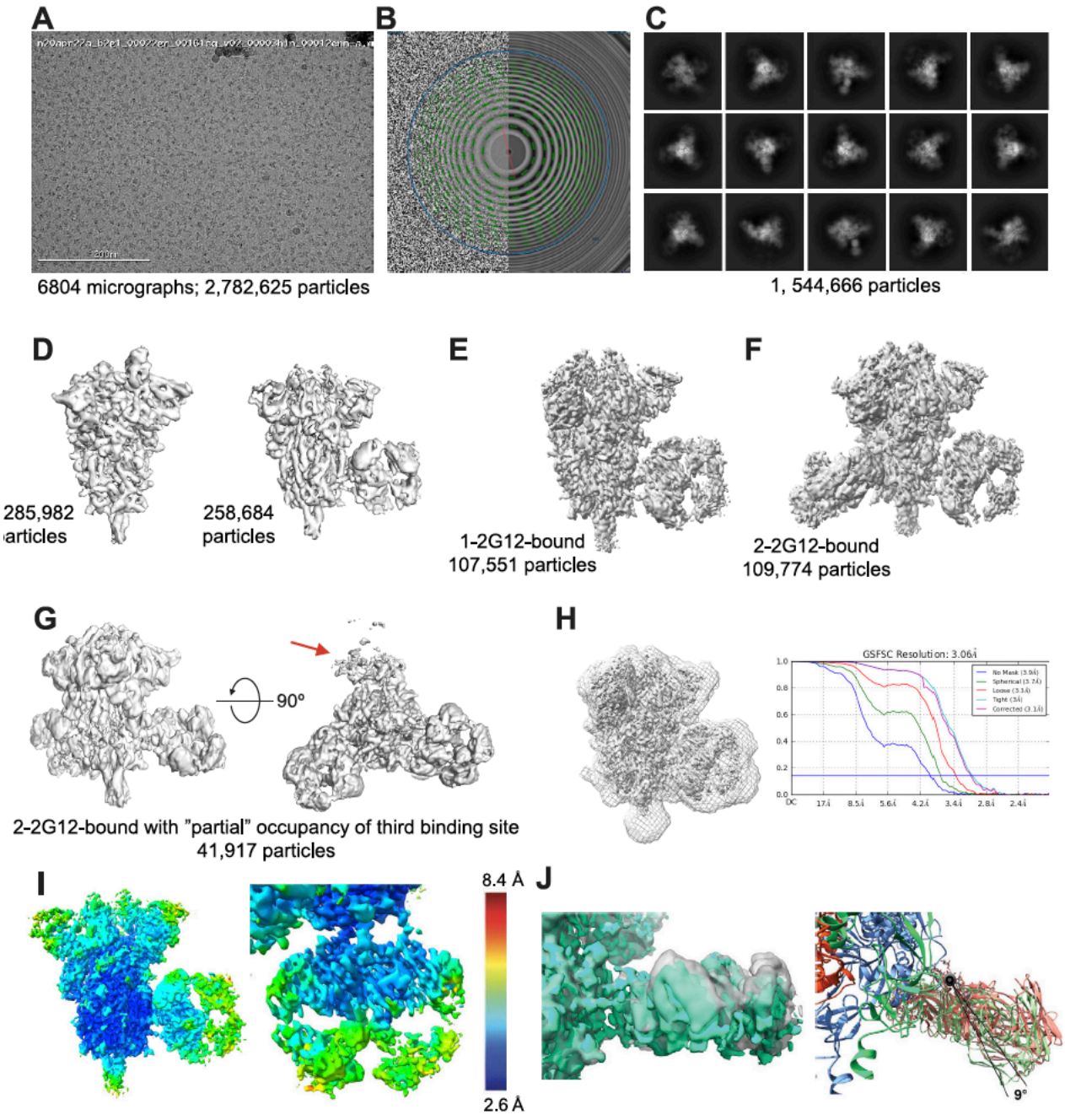

Figure 2. Structural determination of SARS-CoV-2 S protein complex with 2G12.
(A) Representative micrograph. (B) CTF fit (C) Representative 2D class averages. (D) Maps for (left) unliganded and (right) 2G12-bound S obtained after 3 D classification. (E-G) Refined maps for SARS-CoV-2 S protein bound to (E) 1-2G12, (F) 2-2G12-, and (G) 2-2G12 (with partial occupancy at the third binding site) Fab2 molecules. Red arrow in (G) points to disordered 2G12 Fab2 bound at the third binding site. (H) (Left) Map combining all particles and focusing refinement on the region within the masks that is shown as a gray mesh overlaid on the final refined map shown as a gray surface. (Right) Fourier shell correlation curves. (I) (Left) Cryo-EM reconstruction of 2G12 bound to the SARS-CoV-2 spike colored by local resolution. (Right) Zoomed-in view showing the cryo-EM reconstruction of the bound 2G12 Fab. (J) (Left) Two distinct states were resolved from the cryo-EM data by heterogeneous classification. Density for the two observed states were shown in green and gray. (Right) Cartoon representation of the SARS-CoV-2 S-protein (bright green, bright orange, blue) and the two 2G12 orientations. The axis of rotation hinged around glycan 709 is represented by a gray cylinder.