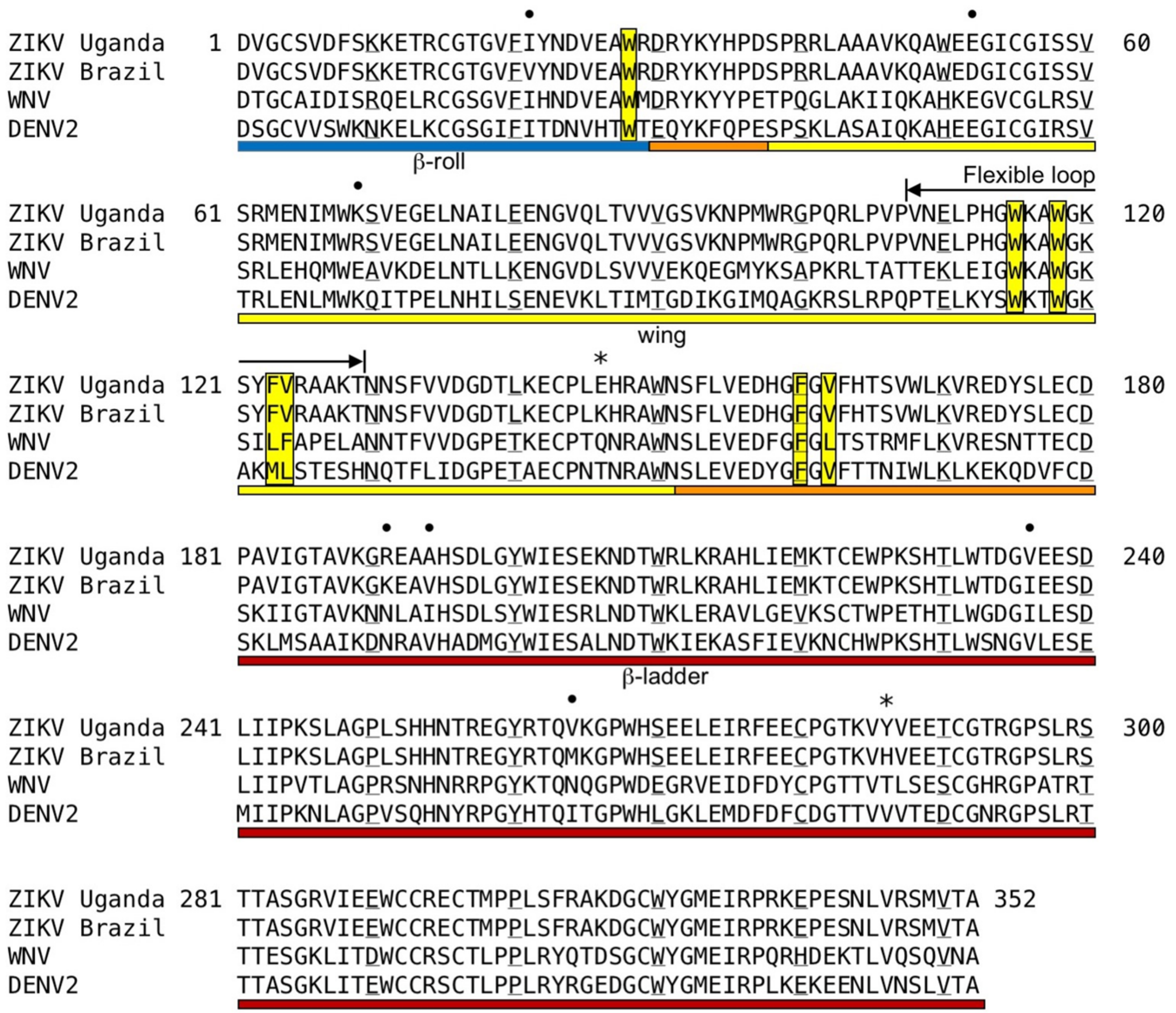
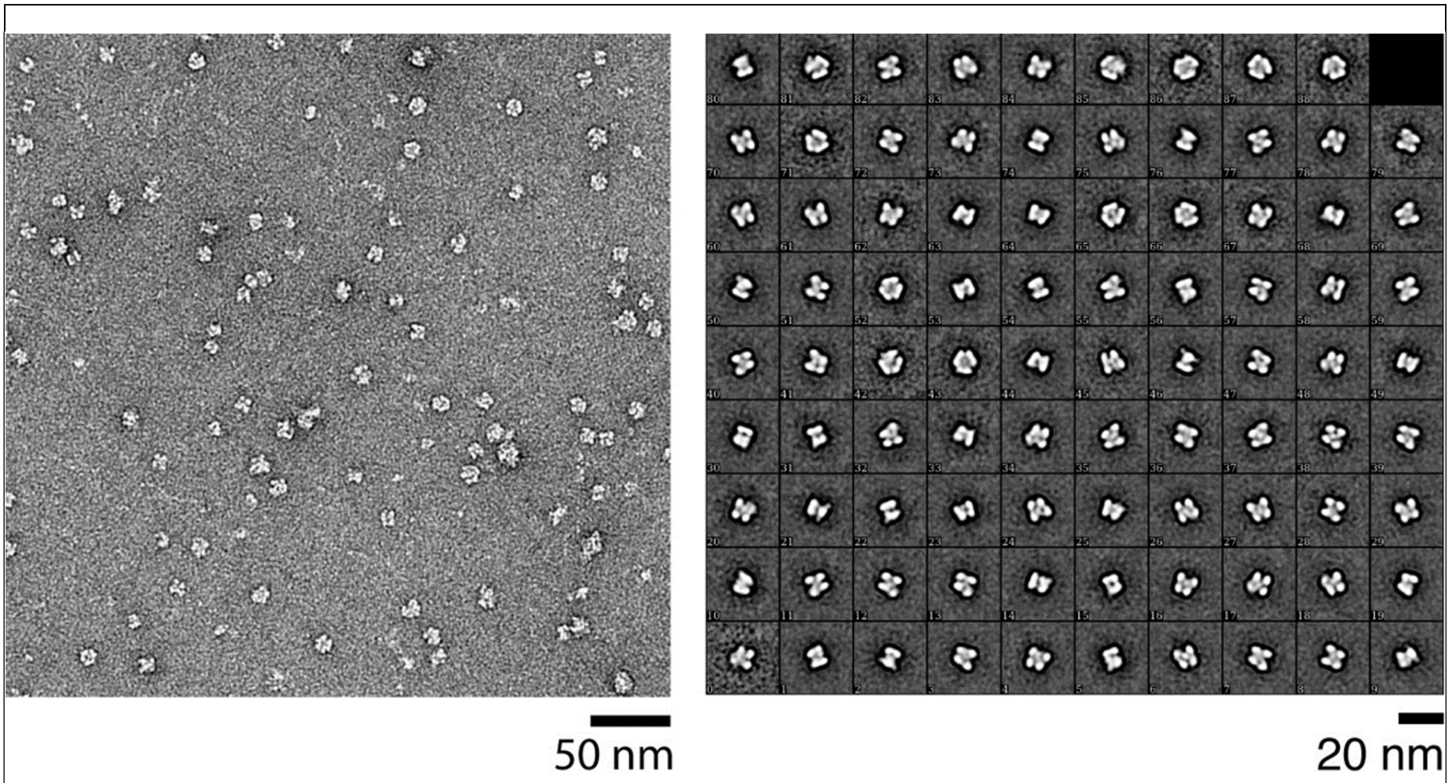


Supplementary Figure 1

Sequence alignment of NS1 proteins

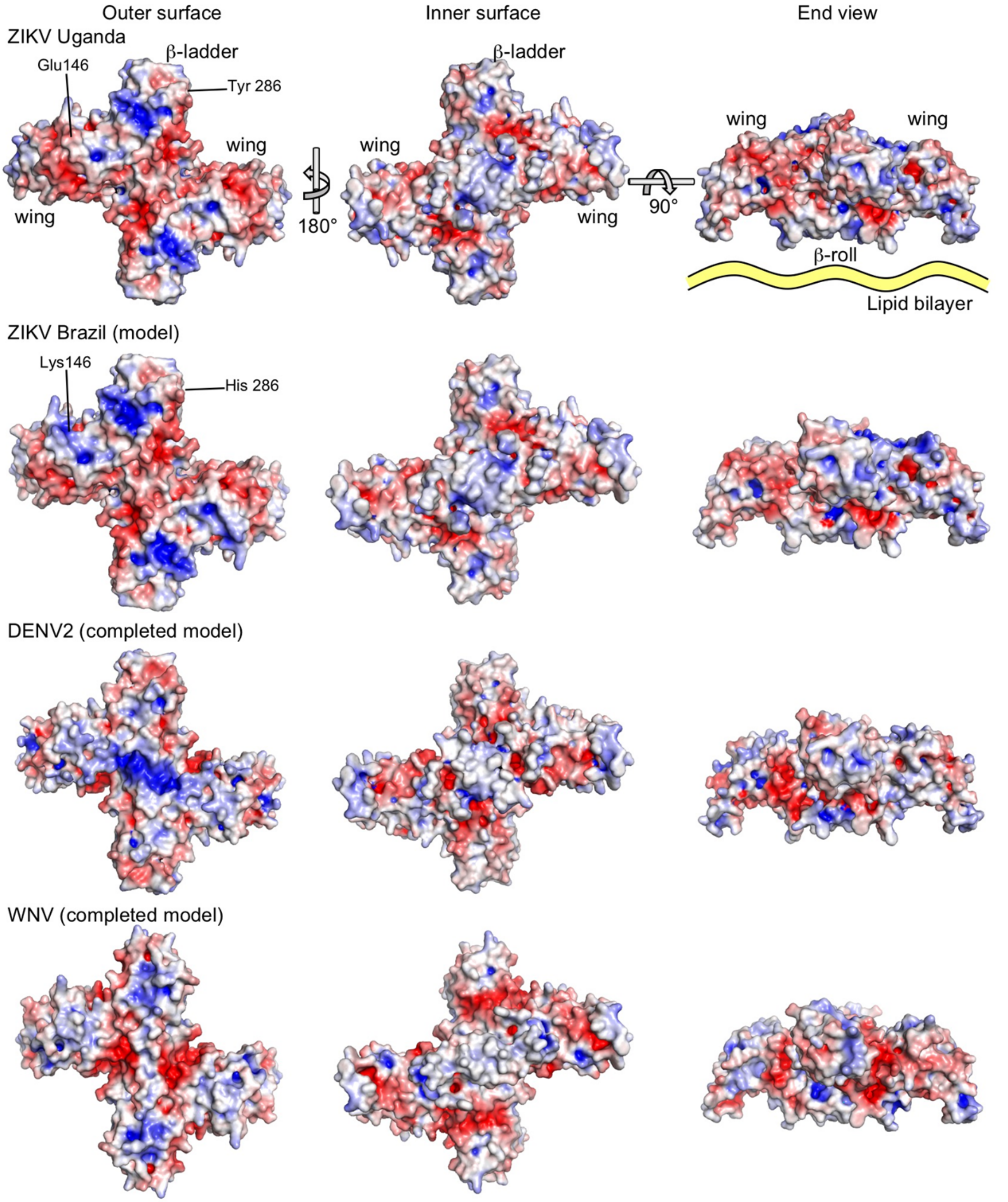
Sequence alignment of NS1 proteins from $\text{ZIKV}_{\text{Uganda}}$ (MR-766), $\text{ZIKV}_{\text{Brazil}}$ (KU49755, identical to H/PF/2013), WNV (NY99) and DENV2 (16681). Hydrophobic residues mentioned in the text are highlighted in yellow. Non-conservative differences between $\text{ZIKV}_{\text{Uganda}}$ and $\text{ZIKV}_{\text{Brazil}}$ are marked with an asterisk and conserved differences with a dot. Colored bars below the alignment represent the β-roll (blue), wing (yellow) with connector sub-domain (orange) and β-ladder (red). The wing domain flexible loop is indicated above the alignment. Every tenth residue is underlined.



Supplementary Figure 2

Negative-stain electron microscopy visualization of the hexamer fraction of ZIKV NS1.

The averages (right) were obtained by classification of 4122 particle projections in raw images such as the representative example at left. Two of the classes from this group are shown in Fig. 3.



Supplementary Figure 3

Variation in surface potential of flavivirus NS1 proteins.

The inner membrane- or lipid-proximal surface (center column) is more hydrophobic (white) than is the outer polar surface (left column). Surfaces are colored by electrostatic potential from -5 kT (red) to +5 kT (blue). On the inner hydrophobic face (right column), the aromatic side chains in the wing flexible loop expand the hydrophobic surface outward from the β-roll at the center of the dimer. For this figure, the NS1 from $\text{ZIKV}_{\text{Brazil}}$ was modeled from ZIKV $\text{NS1}_{\text{Uganda}}$, and the flexible loops were modeled on the DENV2 and WNV NS1 structures based on the $\text{ZIKV}_{\text{Uganda}}$ NS1 structure. The outer polar NS1 surface, which is presented to the host immune proteins, varies greatly among NS1 proteins (left column). Changes in the outer surface are apparent among ZIKV strains by comparison of NS1 proteins from the first-identified 1947 Ugandan strain MR-766, for which the crystal structure is reported (top panel), and from a strain isolated in the 2015 Brazilian outbreak (GenBank KU497555; second panel), which has an identical NS1 sequence to strain H/PF/2013 from the French Polynesian outbreak.