Supplemental Information

Structural Basis for Switch in Receptor Binding Specificity of two H5N1 Hemagglutinin Mutants

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Figure S1. Related to Figures 2 to 5. Sequence comparison of the HA1 domains of H5N1 HAs and an H2 HA[a].

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H3 numbering:                                             10        20        30  34
A/duck/Egypt/10185SS/2010                   MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNV
A/chicken/Vietnam/NCVD-093/2008             MEKIVLLLAIIGLVKSDQICVGYHANNSTEQVDTIMEKNI
A/Vietnam/1203/2004                         MEKIVLLFAIVSLVKSDQICIGYHANNSTEQVDTIMEKNV
A/Indonesia/5/2005                          MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNV
A/Singapore/1/1957(H2N2)                    MAIIYLIL-LFTAVRGDQICIGYHANNSTEKVDTILERNV

H3 numbering:                    35   40        50         60        70        80 83
A/duck/Egypt/10185SS/2010         TVTHAQDILEKTHNGKLCNLDGVKPLIILRDCSVAGWLLGNPMCDEFLNVP
A/chicken/Vietnam/NCVD-093/2008   TVTHAQDILEKTHNGKLCNLNGVKPLIILKDCSVAGWLLGNPMCDEFLNVS
A/Vietnam/1203/2004               TVTHAQDILEKKHNGKLCDLDGVKPLIILRDCSVAGWLLGNPMCDEFINVP
A/Indonesia/5/2005                TVTHAQDILEKTHNGKLCDLDGVKPLIILRDCSVAGWLLGNPMCDEFINVP
A/Singapore/1/1957(H2N2)          TVTHAKDILEKTHNGKLCKLNGIPPLELGDCSIAGWLLGNPECDRLLSVP

H3 numbering:                   83A     90        100       110       120        129
A/duck/Egypt/10185SS/2010         EWSYIVEKINPANDLCYPGNFNDYEELKHLLSRINHFEKIQITPKNSWSD
A/chicken/Vietnam/NCVD-093/2008   EWSYIVEKASPANGLCYPGDFNDYEELKHLLSRINHFEKIKIIPKSYWSN
A/Vietnam/1203/2004               EWSYIVEKANPVNDLCYPGDFNDYEELKHLLSRINHFEKIQIIPKSSWSS
A/Indonesia/5/2005                EWSYIVEKANPTNDLCYPGSFNDYEELKHLLSRINHFEKIQIIPKSSWSD
A/Singapore/1/1957(H2N2)          EWSYIMEKENPRDGLCYPGSFNDYEELKHLLSSVKHFEKVKILPKDRWTQ

H3 numbering:                   130133a    140       150       160       170     178
A/duck/Egypt/10185SS/2010         HEAS-GVSSACPYQGRSSFFRNVVWLTKKDNAYPTIKRSYNNTNQEDLLV
A/chicken/Vietnam/NCVD-093/2008   HETSLGVSSACSYLENPSFFRNVVWLTKKNNTYPPIKVNYTNANQKDLLV
A/Vietnam/1203/2004               HEASLGVSSACPYQGKSSFFRNVVWLIKKNSTYPTIKRSYNNTNQEDLLV
A/Indonesia/5/2005                HEASSGVSSACPYLGSPSFFRNVVWLIKKNSTYPTIKKSYNNTNQEDLLV
A/Singapore/1/1957(H2N2)          HTTT-GGSRACAVSGNPSFFRNMVWLTEKGSNYPVAKGSYNNTSGEQMLI

H3 numbering:                    180       190       200       210       220     228
A/duck/Egypt/10185SS/2010         LWGIHHPNDATEQTRLYQNPTTYISVGTSTLNQKLVPKIATRSKVKGQSG
A/chicken/Vietnam/NCVD-093/2008   LWGIHHPNNEAEQKMIYQNLNTYVSVGTSTLNQRLVPKIATRSKVNGQSG
A/Vietnam/1203/2004               LWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPRIATRSKVNGQSG
A/Indonesia/5/2005                LWGIHHPNDAAEQTRLYQNPTTYISIGTSTLNQRLVPKIATRSKVNGQSG
A/Singapore/1/1957(H2N2)          IWGVHHPNDEKEQRTLYQNVGTYVSVGTSTLNKRSTPDIATRPKVNGLGS

H3 numbering:                    230       240       250       260        270    277
A/duck/Egypt/10185SS/2010         RMEFFWTILKSNDAINFESNGNFIAPENAYKIVKKGDSTIMKSELEYGDC
A/chicken/Vietnam/NCVD-093/2008   RMDFFWTILKPNDTINFDSNGNFIAPEYAYKIVKKGDSAIMKSELEYGNC
A/Vietnam/1203/2004               RMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSTIMKSELEYGNC
A/Indonesia/5/2005                RMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNC
A/Singapore/1/1957(H2N2)          RMEFSWTLLDMWDTINFESTGNLIAPEYGFKISKRGSSGIMKTEGTLENC

H3 numbering:                     280       290       300       310       320
A/duck/Egypt/10185SS/2010         NTKCQTPIGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQGE
A/chicken/Vietnam/NCVD-093/2008   NTKCQTPIGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNAPQIE
A/Vietnam/1203/2004               NTKCQTPMGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQRE
A/Indonesia/5/2005                NTKCQTPMGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQRE
A/Singapore/1/1957(H2N2)          ETKCQTPLGAINTTLPFHNVHPLTIGECPKYVKSEKLVLATGLRNVPQIE
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[a] H5 HA residues that are critical for the changes from avian to human receptor specificity are highlighted in red and compared to the corresponding residues in human H2 HA. Other important H5 HA residues in the receptor binding sites are highlighted in cyan. Yellow indicates positions of T318I and H110Y mutations outside of the receptor binding site that were important for transmission in ferrets in H5 HAs of A/Vietnam/1203/2004 and A/Indonesia/5/2005. The H3 numbering system is used throughout in this paper.

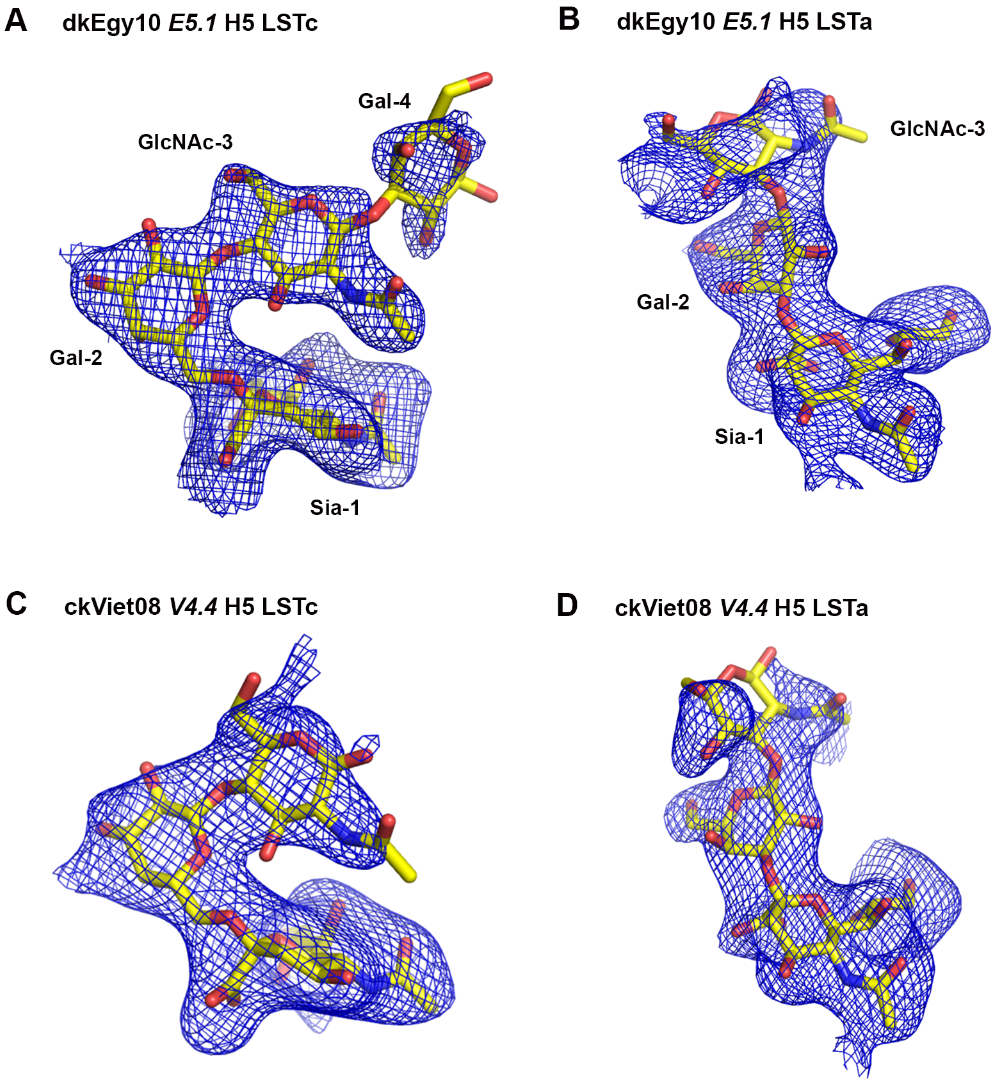


Figure S2. Related to Figure 1. Simulated annealing omit (Fo-Fc) electron density maps of glycan ligands bound to H5 HAs. (A) LSTc bound to *E*5.1 HA (2.7 Å resolution). (B) LSTa bound to *E*5.1 HA (2.6 Å resolution). (C) LSTc bound to *V*4.4 HA (2.7 Å resolution). (D) LSTa bound to *V*4.4 HA (2.7 Å resolution). The electron density for the glycan receptors is represented in a blue mesh and contoured at 2.0 σ.

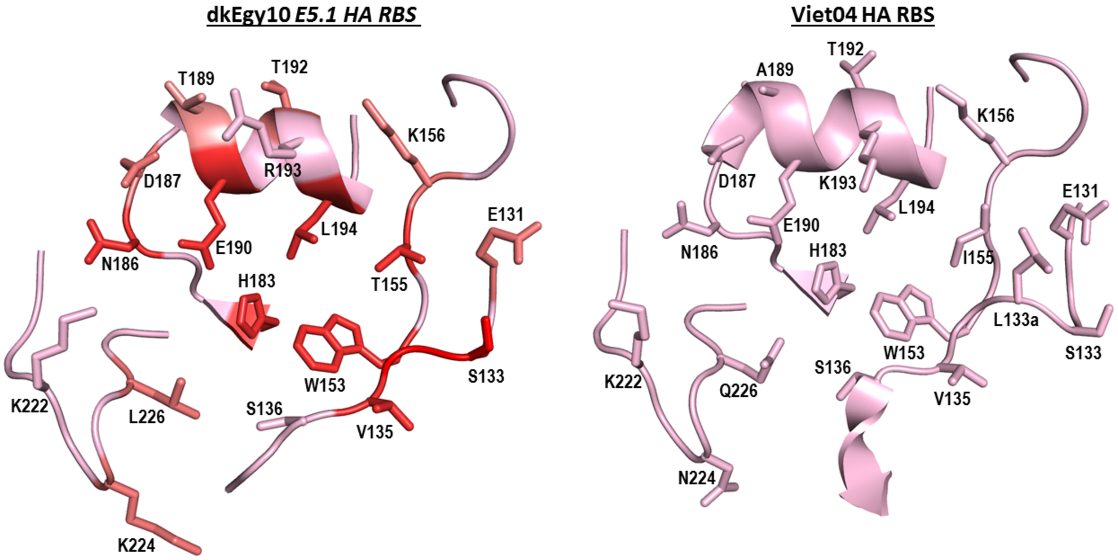
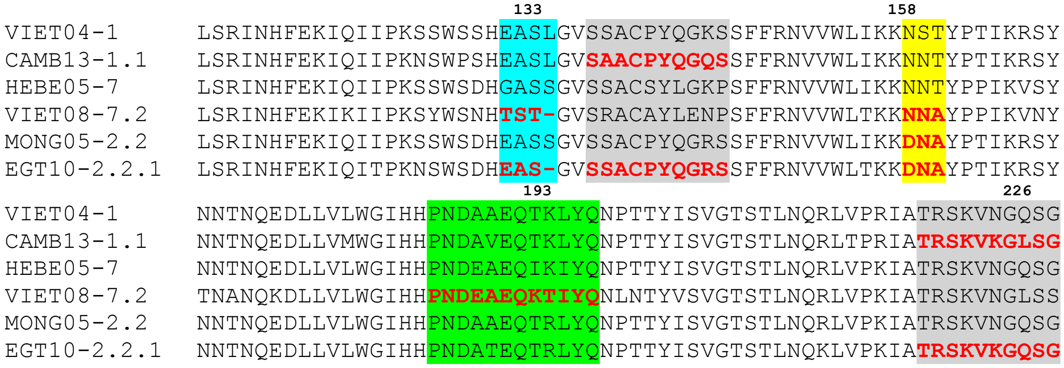


Figure S3. Related to Figures 2 to 5. Calculation of network properties of key RBS residues in *E*5.1 HA.

The RBS of *E*5.1 HA is shown on the left and key residues are labeled and shaded from light to dark red. The darker color represents closer similarity of network property of that residue to the corresponding residue in RBS of human H2 HA. On the right is the RBS of Viet04 HA, which did not acquire any of the 4 molecular features and, hence, has the lowest similarity in terms of network properties with RBS of human H2 HA. Note that residues in *E*5.1 HA RBS share a closer similarity in terms of network properties to human H2 HA than their identical counterparts in Viet04 H5 HA RBS.



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                                                133                                    158
VIET04-1       LSRINHFEKIQIIPKSSWSSHEASLGVSSACPYQGKSSFFRNVVWLIKKNSTYPTIKRSY
CAMB13-1.1     LSRINHFEKIQIIPKNSWPSHEASLGVSAACPYQGQSSFFRNVVWLIKKNNTYPTIKRSY
HEBE05-7       LSRINHFEKIQIIPKSSWSDHGASSGVSSACSYLGKPSFFRNVVWLIKKNNTYPPIKVSY
VIET08-7.2     LSRINHFEKIKIIPKSYWSNHTST-GVSRACAYLENPSFFRNVVWLTKKNNAYPPIKVNY
MONG05-2.2     LSRINHFEKIQIIPKSSWSDHEASSGVSSACPYQGRSSFFRNVVWLIKKDNAYPTIKRSY
EGT10-2.2.1    LSRINHFEKIQITPKNSWSDHEAS-GVSSACPYQGRSSFFRNVVWLTKKDNAYPTIKRSY
                                                 193                                       226
VIET04-1       NNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPRIATRSKVNGQSG
CAMB13-1.1     NNTNQEDLLVMWGIHHPNDAVEQTKLYQNPTTYISVGTSTLNQRLTPRIATRSKVKGLSG
HEBE05-7       NNTNQEDLLVLWGIHHPNDEAEQIKIYQNPTTYISVGTSTLNQRLVPKIATRSKVNGQSG
VIET08-7.2     TNANQKDLLVLWGIHHPNDEAEQKTIYQNLNTYVSVGTSTLNQRLVPKIATRSKVNGLSS
MONG05-2.2     NNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISVGTSTLNQRLVPKIATRSKVNGQSG
EGT10-2.2.1    NNTNQEDLLVLWGIHHPNDATEQTRLYQNPTTYISVGTSTLNQKLVPKIATRSKVKGQSG
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Abbreviation	**H5 clade**	**Strain name**
VIET04	1	A/Vietnam/1203/2004
CAMB13	1.1	A/Cambodia/X0810301/2013
HEBE05	7	A/chicken/Hebei/326/2005
VIET08	7.2	A/chicken/Vietnam/NCVD-093/08
MONG05	2.2	A/whooper swan/Mongolia/244/2005
EGT10	2.2.1	A/duck/Egypt/10185SS/2010

Figure S4. Related to Figures 2 to 5. Evolution of RBS features in representative H5 clades. Shown below is a sequence alignment of the RBS-spanning region (residues 108-228) from H5 clades discussed in the main text. For each clade, a strain representative of a recent RBS variant and an older prototype strain are shown. Amino acids that contribute to features 1, 2, 3 and 4 are highlighted in cyan, grey, green and yellow, respectively. RBS features that have evolved to match human-adapted H2 HA are shown in red. Details of the HAs employed in the alignment are shown in the table above.