# Supporting Information

Lin et al. 10.1073/pnas.1218841110
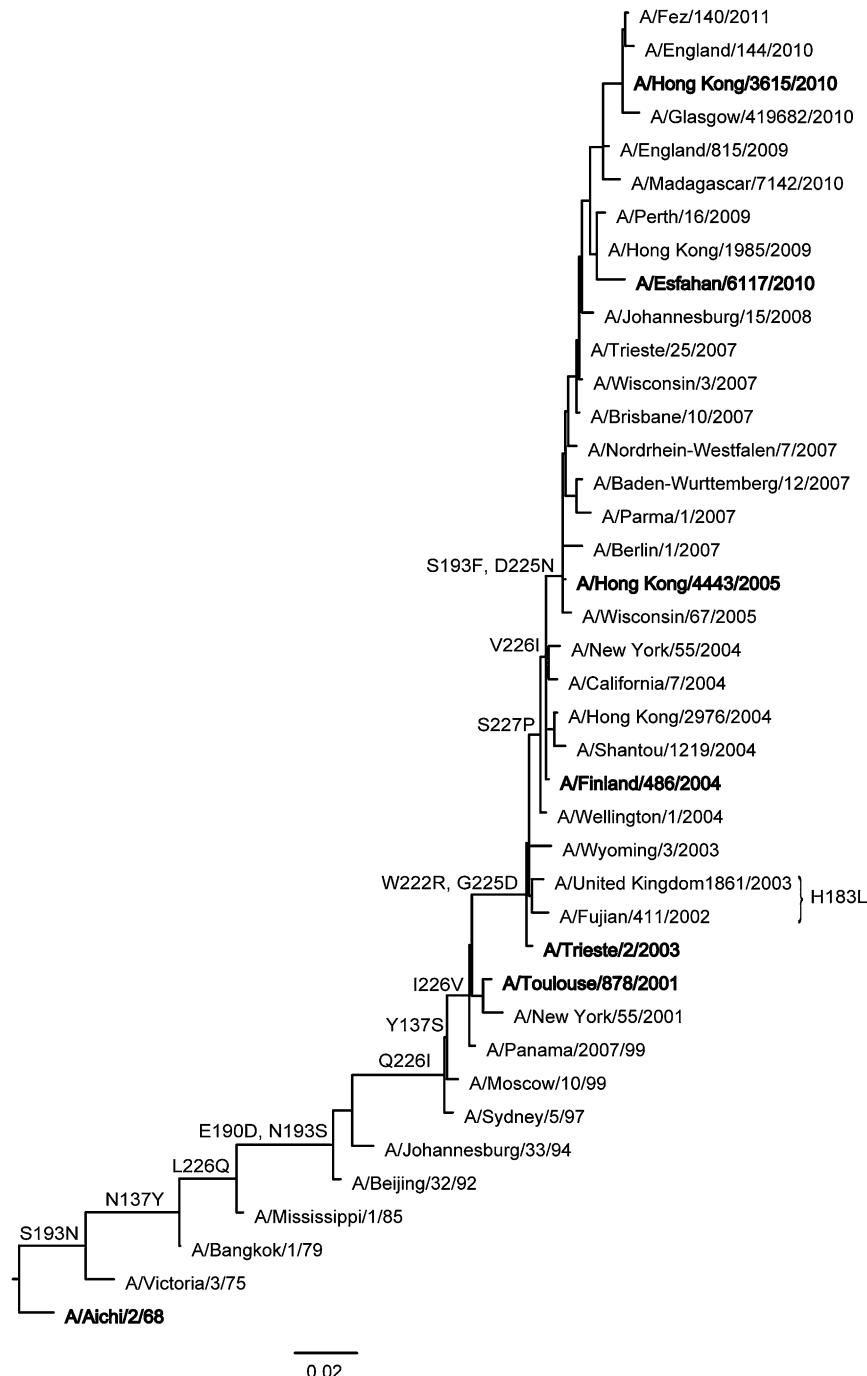
Fig. S1. Phylogenetic analysis of the HA gene sequences of representative H3N2 viruses collected since 1968 with an emphasis on the 1999–2010 period. Analysis was performed using RAxML (1), and selected amino acid substitutions in the vicinity of the HA receptor binding site, as mentioned in the main text, are indicated. The viruses used in this study are shown in bold type, and the scale bar represents 20 nucleotide substitutions/1,000.

1. Stamatakis A, Ludwig T, Meier H (2005) RAxML-III: A fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics* 21(4):456–463.
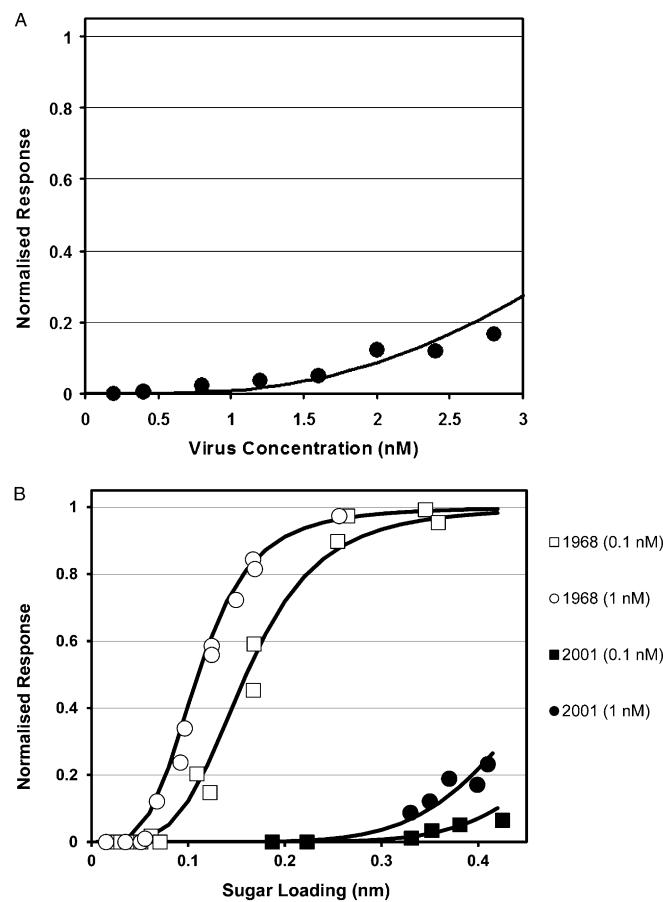
Fig. S2. (*A*) Biolayer-interferometry binding curve of increasing concentrations of A/Esfahan/6117/2010 virus to the human receptor analog, α2,6-sialyl lactosamine, bound to the sensor chip at a loading of 0.38 nm. (*B*) Biolayer-interferometry binding curves of influenza viruses to the avian receptor analog, α2,3-sialyl lactosamine, bound to the sensor chip. Viruses used are indicated 1968, X31 (open symbols), and 2001, A/Toulouse/878/2001 (filled symbols), at virus concentrations of 0.1 nM (squares) and 1 nM (circles).

Fig. S3. Overlay of the C-α traces of H3 HAs: 1968 (purple, HA1/2), 2004 (green, HA0), and 2005 (blue, HA0). The rmsd of 2004 and 2005 HAs to 1968 H3 HA are 0.680 and 0.600 Å, respectively. The three backbone structures are remarkably similar except in the region of the HA2 fusion peptide (internalized in the 1968 HA1/2 structure).
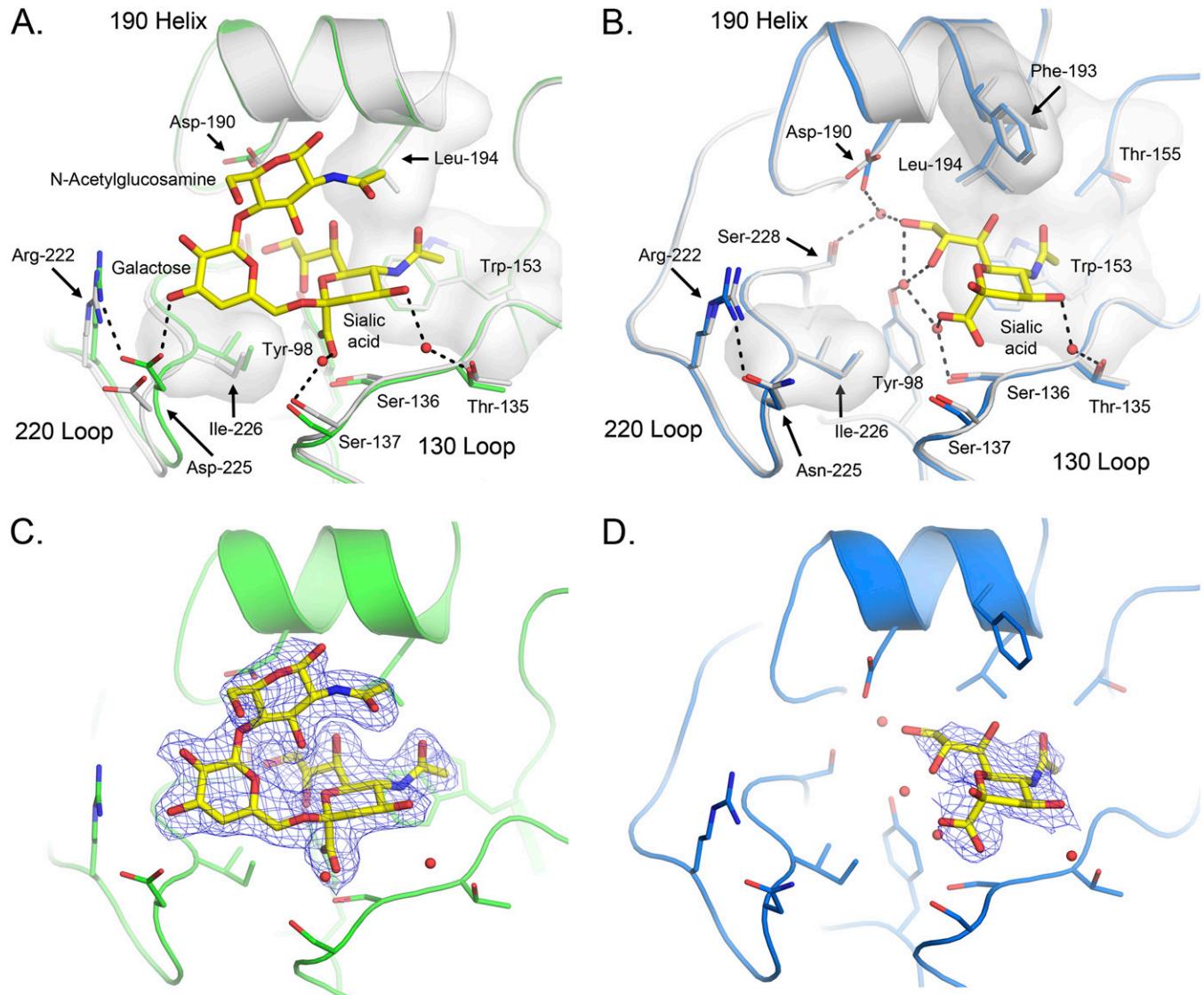
Fig. S4. Electron density maps of the receptor analogs in the complexes shown in Fig. 3 *A* and *B*. Fig. 3 *A* and *B* are reproduced (as *A* and *B*) and beneath them the 2Fo-Fc density maps (*C* and *D*). The density is contoured at 1-s level above the mean (*C*) and at 0.8 s (*D*).

Table S1. Agglutination of SA2,3 and SA2,6 on RBCs from different species by *Maackia amurensis* II (MAAII) and *Sambucus nigra* (SNA) lectins

Lectin	Chicken	Turkey	Guinea pig
MAA II (SA 2,3)	10,240	10,240	5,120
SNA (SA 2,6)	10,240	40,960	163,840
Relative SNA/MAA II binding	1	4	32

Hemagglutination titers were determined using 1% (vol/vol) RBCs from various species.

Table S2. Statistics of crystallographic data

Crystallographic parameters	Hemagglutinin/receptor analog							
	1968 X31	2004 Finland				2005 Hong Kong		
	LSTc	APO	LSTc	2,6SLN	2,3SLN	APO	2,6SLN	2,3SLN
Data collection								
X-ray source	DIAMOND IO4	DIAMOND IO3	DIAMOND IO3	DIAMOND IO4	DIAMOND IO4	DIAMOND IO3	DIAMOND IO4	DIAMOND IO4
Wavelength, Å	0.9795	0.9763	0.9763	0.9795	0.9795	0.9763	0.9795	0.9795
Space group	$P4_1$	H32					H32	
$a = b$, Å	160.77	100.92	101.21	101.13	100.86	100.89	100.92	100.97
c, Å	177.21	387.18	387.94	387.88	386.53	386.26	386.69	387.19
α, β, γ (°)	90, 90, 90	90, 90, 120					90, 90, 120	
Resolution, Å	177.21–2.85 (2.95–2.85)	64.87–1.90 (2.00–1.90)	65.03–1.85 (1.95–1.85)	47.09–1.88 (1.95–1.88)	46.96–1.79 (1.89–1.79)	64.79–1.85 (1.95–1.85)	46.99–1.80 (1.9–1.80)	46.96–1.79 (1.89–1.79)
Observations	628,482 (91,853)	413,455 (51,271)	582,750 (85,771)	449,376 (65,943)	516,517 (74,885)	552,054 (75,322)	504,376 (73,631)	507,798 (73,742)
Unique observations	104,897 (15,277)	60,416 (8,709)	66,050 (9,510)	62,699 (9,058)	71,503 (10,309)	65,292 (9,430)	70,771 (10,208)	71,580 (10,316)
R_{merger} %	12.8 (66.1)	7.6 (51.7)	8.1 (68.7)	13.3 (60.9)	11.1 (58.3)	12.3 (68.8)	10.1 (64.4)	11.1 (58.3)
I/σ	11.2 (2.7)	13.5 (3.3)	14.1 (3.5)	9.7 (3.0)	11.5 (3.2)	8.3 (2.2)	12.4 (3.0)	11.5 (3.2)
Completeness, %	100 (100)	100 (100)	100 (99.9)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)
Multiplicity	6.0 (6.0)	6.8 (5.9)	8.8 (9.0)	7.2 (7.3)	7.2 (7.3)	8.5 (8.0)	7.1 (7.2)	7.2 (7.3)
Refinement								
No. reflections	99,611	57,367	62,701	59,519	67,881	61,939	67,196	67,956
R, %	17.70	17.54	17.64	17.18	16.95	17.95	17.07	16.96
R_{free}, %	19.04	19.43	20.52	19.59	19.21	20.97	19.47	19.14
No. atoms								
Protein (with glycosylation)	11,951	4,041	4,003	4,017	4,017	4,057	4,058	4,039
Receptor analog	182	—	68	46	22	—	21	22
Ligand/ion	169	56	41	41	56	41	56	56
Water	433	428	458	530	536	424	509	584
B factors, $Å^2$								
Protein (with glycosylation)	45.63	34.39	38.74	31.11	27.44	36.83	29.52	26.69
Receptor analog	102.74	—	69.25	42.66	50.88	—	75.95	59.92
Ligand/ion	85.24	67.15	72.82	63.83	52.07	72.69	60.34	54.04
Water	33.52	40.93	46.83	39.47	35.61	42.59	37.71	35.73
Ramachandran								
% Favored	96.44	96.91	96.50	96.50	96.71	96.70	96.70	97.11
% Outliers	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
rmsd								
Bond length, Å	0.0073	0.0094	0.0096	0.0091	0.0082	0.0103	0.0089	0.0095
Bond angle, °	1.4616	1.3535	1.3139	1.3097	1.4153	1.4063	1.3093	1.3775
PDB ID code	2YPG	2YP2	2YP4	2YP3	2YP5	2YP7	2YP8	2YP9

Table S3. GISAID accession numbers of HA genes used in this study

Virus	Accession no.
X31 (A/Aichi/2/68)	EPI242151
A/Toulouse/878/2001	EPI397686
A/Trieste/2/2003	EPI397687
A/Finland/486/2004	EPI397685
A/Hong Kong/4443/2005	EPI397688
A/Esfahan/6117/2010	EPI302190
A/Hong Kong/3615/2010	EPI287110

GISAID, Global Initiative on Sharing All Influenza Data (http://platform.gisaid.org/epi3).