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Supplementary Information for

Universal stabilization of the Influenza Hemagglutinin by structure-based redesign of the pH-switch regions.

Fin J. Milder[1*], Mandy Jongeneelen[1*], Tina Ritschel[1*], Pascale Bouchier[1], Ilona J.M. Bisschop[1], Martijn de Man[1], Daniel Veldman[1], Lam Le[1], Baerbel Kaufmann[1], Mark J. G. Bakkers[1], Jarek Juraszek[1], Boerries Brandenburg[1$], Johannes P. M. Langedijk[1$#]

[1] Janssen Vaccines & Prevention BV, Leiden, The Netherlands

* Authors contributed equally
$ These authors jointly supervised this work: Boerries Brandenburg, Johannes P. M. Langedijk

#Correspondence to:
Johannes P.M. Langedijk
Email: hlangedi@its.jnj.com

This PDF file includes:

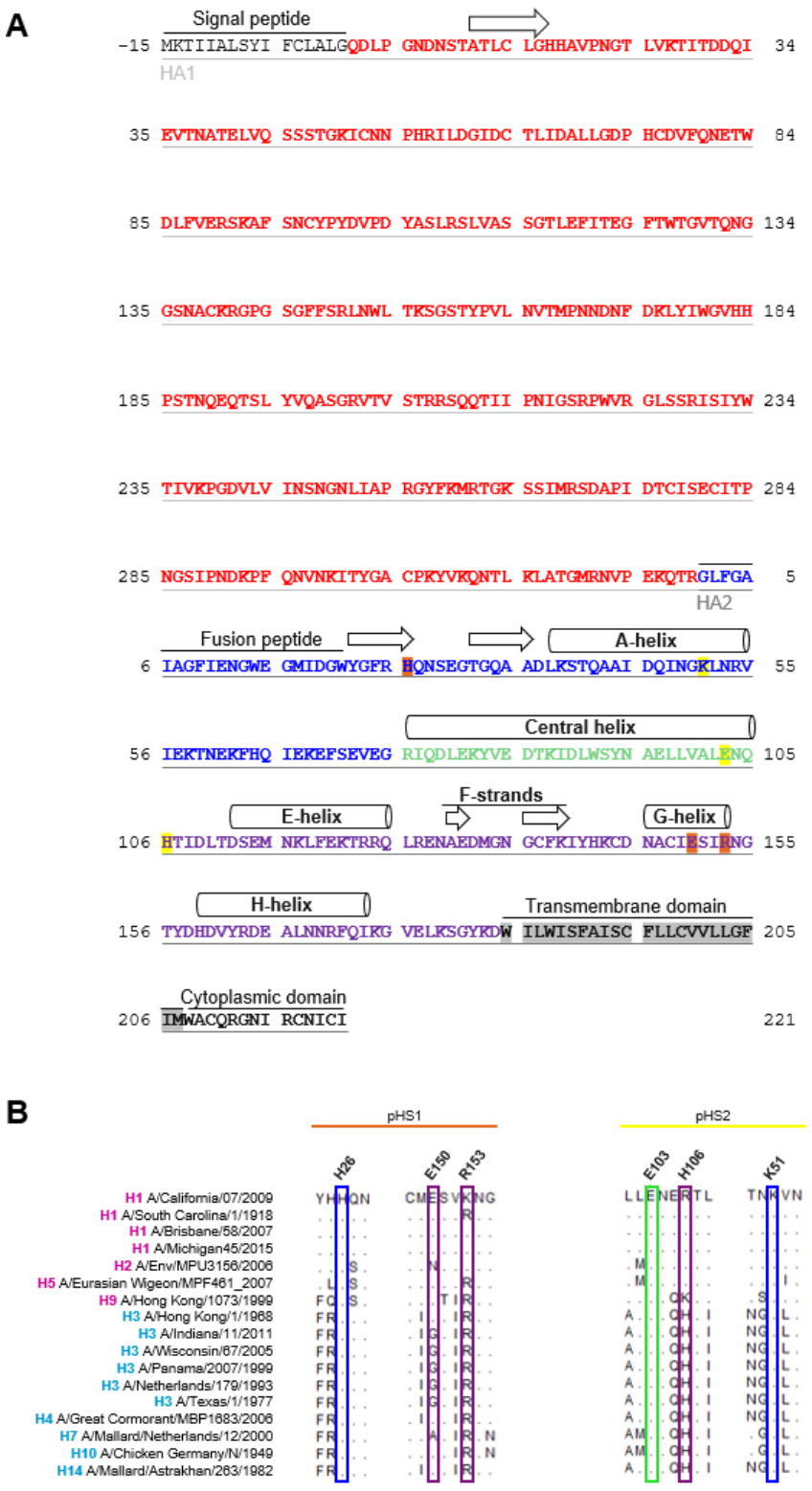
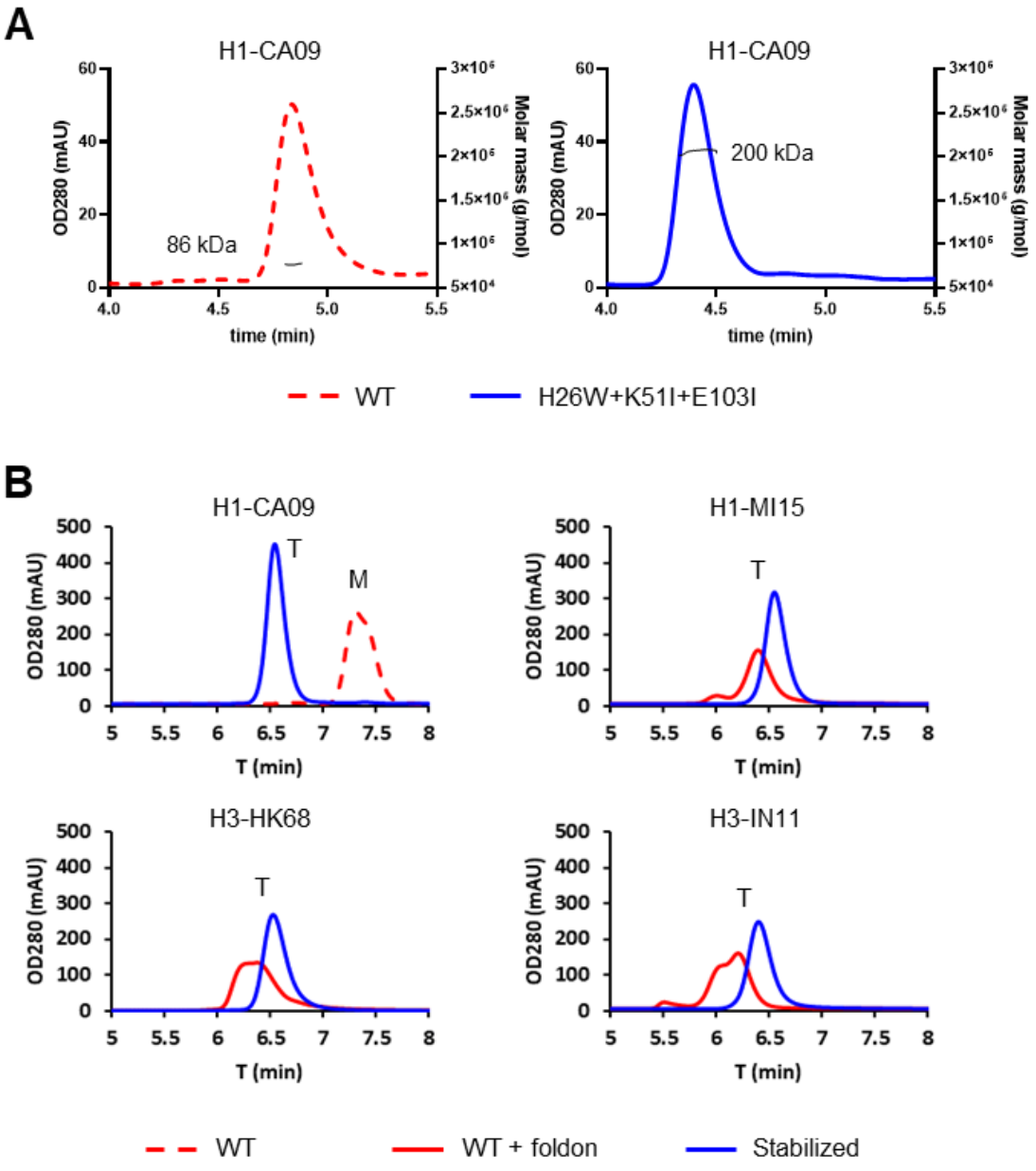


Fig. S1. pH switches 1 and 2 in Flu A HA's. (**a**) Sequence of Full-Length H3-HK68 HA; indicated are the structural elements as highlighted in Figure 1. (**b**) Alignment of the pHS1 and pHS2 residues of Group 1 and 2 HA's. Shown are the sequences of the strains as in Figure 4A with highlighted the histidines and charged residues. Color coding according to Figure 1.


A
H1-CA09
OD280 (mAU)
Molar mass (g/mol)
time (min)
86 kDa
H1-CA09
200 kDa
WT
H26W+K51I+E103I
B
H1-CA09
T
M
H1-MI15
T
H3-HK68
T
H3-IN11
T
OD280 (mAU)
T (min)
WT
WT + foldon
Stabilized

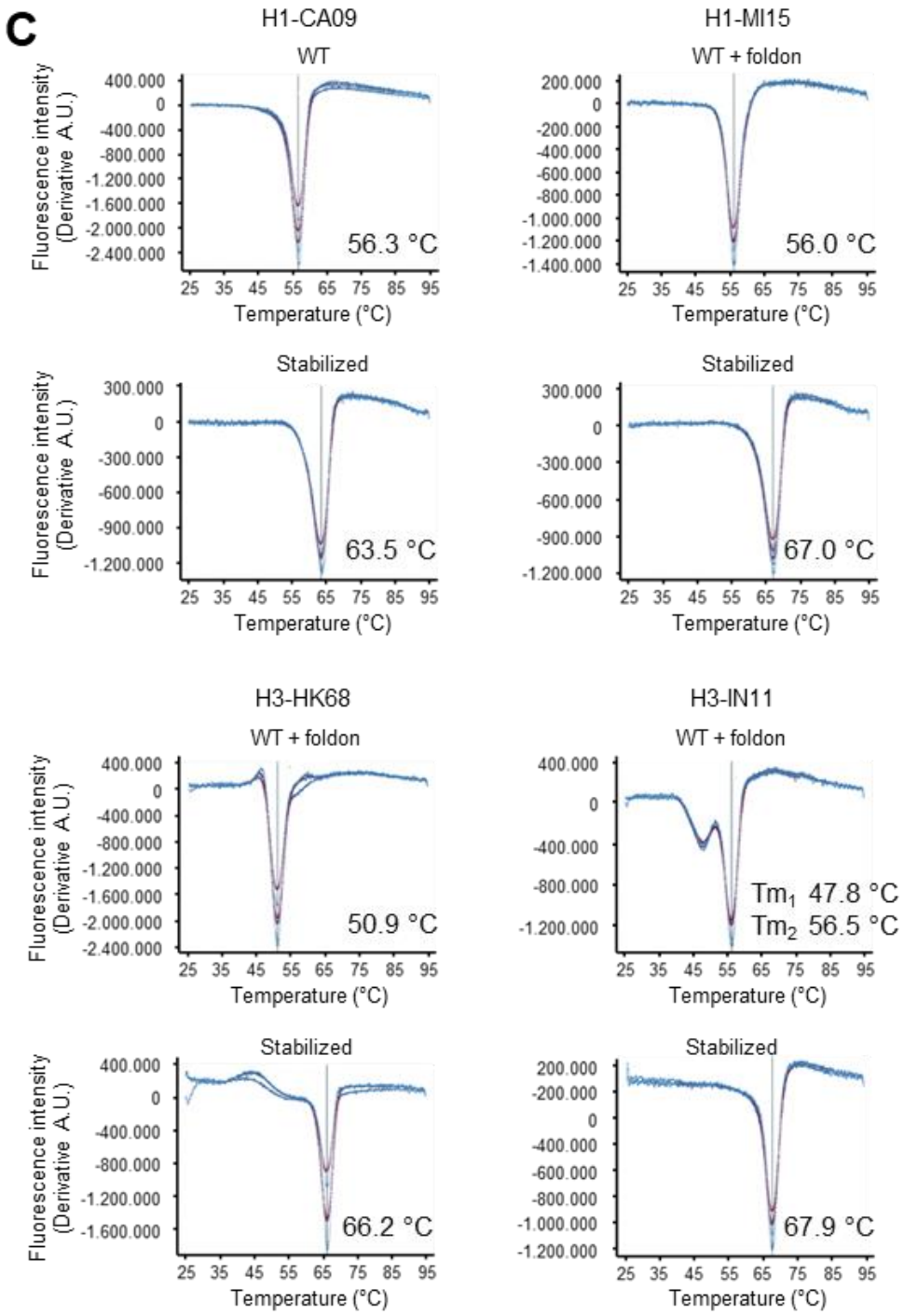


Fig. S2. Comparison of purified stabilized HA and WT with the C-terminal foldon. (**a**) SEC-MALS analysis of crude culture supernatants of Expi293F cells transfected with plasmids encoding WT and stabilized H1-CA09 HA. The black lines show the molar mass traces (right axis). (**b**) Analytical SEC profile of WT and stabilized purified HA protein for H1-CA09, H1-MI15, H3-HK68, and H3-IN11. The monomer and trimer peak are indicated by an M and T, respectively. (**c**) Analysis of melting temperature using differential scanning fluorimetry. The first order derivatives are plotted. Shown are the averaged duplicate runs and Tm determined as the lowest derivative value representing the Tm_{50} value.

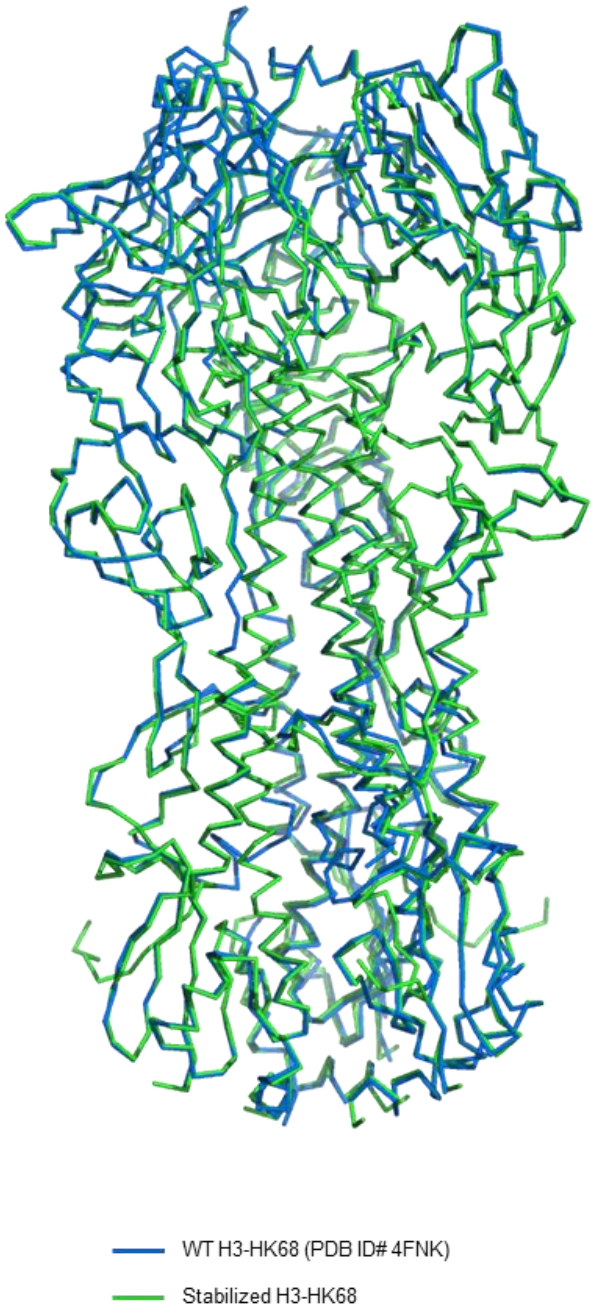


Fig. S3. Comparison of stabilized HA and WT structure. Overlay of the Cα atom traces of stabilized and WT (PDB ID 4FNK, (1)) H3-HK68 HA structures.

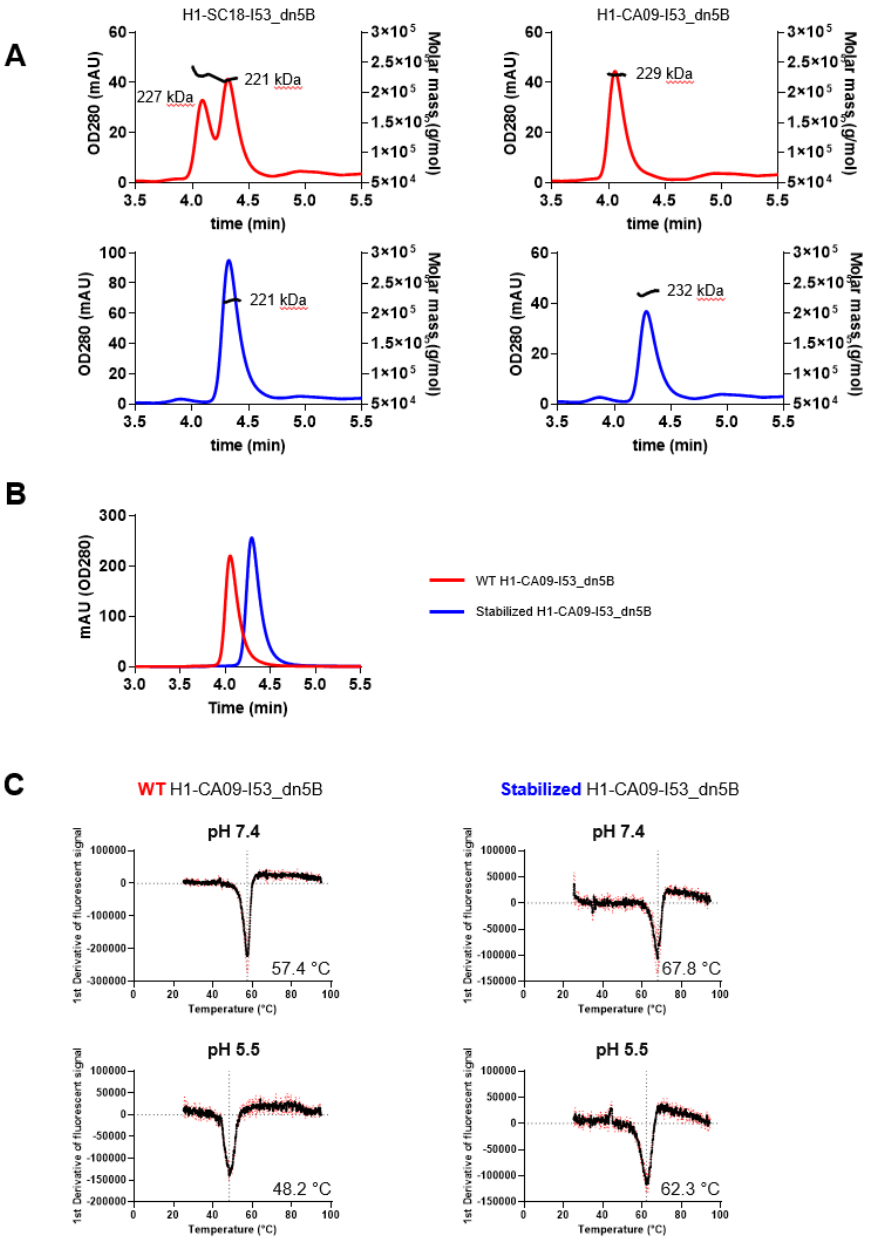


Fig. S4. Biophysical characterization of stabilized and WT H1-SC18-I53_dn5B and H1-CA09-I53_dn5B. (**a**) SEC-MALS analysis of crude culture supernatants of Expi293F cells transfected with plasmids encoding WT and stabilized HA's. The black lines show the molar mass traces (right axis). (**b**) Analytical SEC profile of purified WT and stabilized H1-CA09-I53_dn5B. (**c**) Analysis of melting temperature Tm using differential scanning fluorimetry of purified WT and stabilized H1-CA09-I53_dn5B. Plotted are the averaged first order derivatives of a triplicate run with the lowest derivative value representing the Tm_{50} value.

Table S1. Antigenicity of stabilized and WT HA as determined by ELISA. Shown are the EC_{50} binding values for mAbs CR6261 (group 1 specific, (2)), CR8020 (group 2 specific, (3)), CR9114 (4) and CT149 ((5)) (both group 1 and group 2 binders).

HA	EC50 values (nM)				
		CR6261	**CR8020**	**CR9114**	**CT149**
H1-CA09	WT	1.0 ± 0.1	>70	1.3 ± 0.2	1.6 ± 0.1
	Stabilized	1.8 ± 0.2	>70	1.4 ± 0.1	0.9 ± 0.2
H1-MI15	WT + foldon	0.6 ± 0.1	>70	0.9 ± 0.2	0.4 ± 0.1
	Stabilized	1.3 ± 0.2	>70	1.6 ± 0.1	0.9 ± 0.1
H3-HK68	WT + foldon	>70	1.6 ± 0.1	12.7 ± 0.3.5	0.9 ± 0.2
	Stabilized	>70	1.9 ± 0.1	3.0 ± 0.1	1.1 ± 0.1
H3-IN11	WT + foldon	>70	1.9 ± 0.1	25.0	0.7 ± 0.5
	Stabilized	>70	1.7 ± 0.4	2.8 ± 1.1	0.7 ± 0.1

Table S2. Data collection and processing statistics

X-ray source	PXII/X10SA (SLS[1])
Wavelength (Å)	0.9999
Detector	EIGER
Temperature	100
Space group	I 2_1 3
Cell: a; b; c; (Å) α; β; γ (°)	154.09; 154.09; 154.09 90.0; 90.0; 90.0
Resolution (Å)	2.19 (2.23-2.19)
Unique reflections	31315 (1543)
Completeness (%)	25.6 (26.2)
R_{pim} (%)[6]	100.0 (100.0)
R_{sym} (%)[3]	1.7 (59.1)
R_{meas} (%)[4]	9.5 (297.7)
CC1/2 (%)	100.00 (54.10)
Mean (I)/sd[5]	24.9 (1.3)

[1] SWISS LIGHT SOURCE (SLS, Villigen, Switzerland)

[2] values in parenthesis refer to the highest resolution bin.

[3] $Rsym = \frac{\sum_h \sum_i^{n_h} |\hat{I}_h - I_{h,i}|}{\sum_h \sum_i^{n_h} I_{h,i}}$ with $\hat{I}_h = \frac{1}{n_h} \sum_i^{n_h} I_{h,i}$

where $I_{h,i}$ is the intensity value of the *i*th measurement of h

[4] $Rmeas = \frac{\sum_h \sqrt{\frac{n_h}{n_h - 1}} \sum_i^{n_h} |\hat{I}_h - I_{h,i}|}{\sum_h \sum_i^{n_h} I_{h,i}}$ with $\hat{I}_h = \frac{1}{n_h} \sum_i^{n_h} I_{h,i}$

where $I_{h,i}$ is the intensity value of the *i*th measurement of h

[5] calculated from independent reflections

[6] Precision-indicating $Rpim = \frac{\sum_h \sqrt{1/(N-1)} |\hat{I}_h - \langle I_h \rangle|}{\sum_h \langle I_h \rangle}$

Table S3. Refinement statistics[1]

Resolution (Å)	108.96 - 2.19
Number of reflections (working/test)	29737 / 1577
R_{cryst} (%)	19.8
R_{free} (%)[2]	25.1
Total number of atoms: Protein Water β-D-mannose α-D-mannose N-acetyl-D-glucosamine Nitrate	3853 134 22 22 126 4
Deviation from ideal geometry:[3] Bond lengths (Å) Bond angles (°) Bonded B's (Å^2)[4]	0.004 1.41 4.5
Ramachandra plot: [5] Most favored regions (%) Additional allowed regions (%) Generously allowed regions (%) Disallowed regions (%)	89.9 8.9 0.7 0.5

[1]Values as defined in REFMAC5(6), without sigma cut-off
[2]Test-set contains 5.0% of measured reflections
[3]Root mean square deviations from geometric target values
[4]Calculated with MOLEMAN (7)
[5]Calulated with PROCHECK (8)

Table S4. Identification of rare mutations in H3 HA sequences of repaired strains.

Table S5. SEC-MALS analysis Position	Domain	**H3 Consensus amino acid**	Minimum frequency	A/Netherlands/179/1993	Frequency [%]	A/Wisconsin/67/2005	Frequency [%]	A/Brisbane/10/2007	Frequency [%]	A/Singapore/INFIMH/16/0019/2016	Frequency [%]
122	HA1	**N**	1.4	N	67.4	D	1.4	N	67.4	N	67.4
135	HA1	**T**	2.8	K	2.8	T	61.7	T	61.7	T	61.7
138	HA1	**A**	0.9	**T**	**0.9**	S	8	A	90.7	A	90.7
186	HA1	**G**	0.9	S	27.4	**V**	**0.9**	G	69.9	G	69.9
194	HA1	**L**	0.4	L	98.8	L	98.8	**P**	**0.4**	**P**	**0.4**

Table S5. SEC-MALS analysis

H1-CA09-I53_dn5B	Peak (trimer)			
	Mw *(kDa)*	Rh *(nm)*	Mass fraction *(%)*	Retention time *(min)*
WT	233.6	7.7	100	4.065
Stabilized	232.6	6.8	100	4.303

SI References

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