SUPPLEMENTARY MATERIALS

Discovery of a novel tetrapeptide against Influenza A virus: rational design, synthesis, bioactivity evaluation and computational studies.

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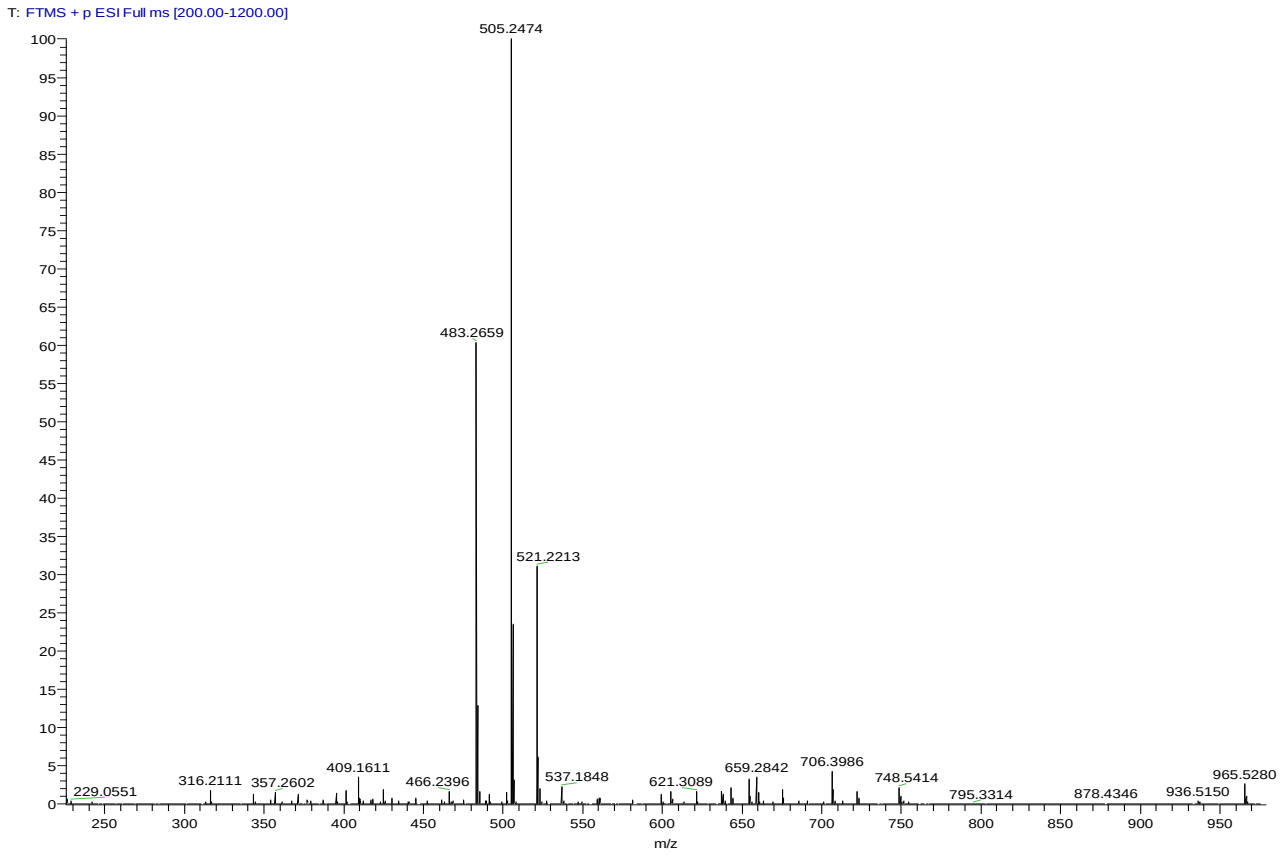
Supplementary schemes and figures:

Table S1. Analytical data of peptides **3-10**.

Pep.	Sequence	HPLC k'[a]	HRMS
1[a]	SKHS	4.68	499.26235
3	AKHS	1.10	483.2659
4	SAHS	0.54	443.1667
5	SKAS	1.85	434.2055
6	SKHA	4.37	483.2654
2[a]	SLDC	3.93	477.18970
7	ALDC	5.67	462.2003
8	SADC	3.50	436.1484
9	SLAC	5.16	434.2050
10	SLDA	5.93	446.2235

[a] k'=[(peptide retention time-solvent retention time)/solvent retention time].

Supplementary figures of Mass spectrometry and HPLC of peptides used in the study



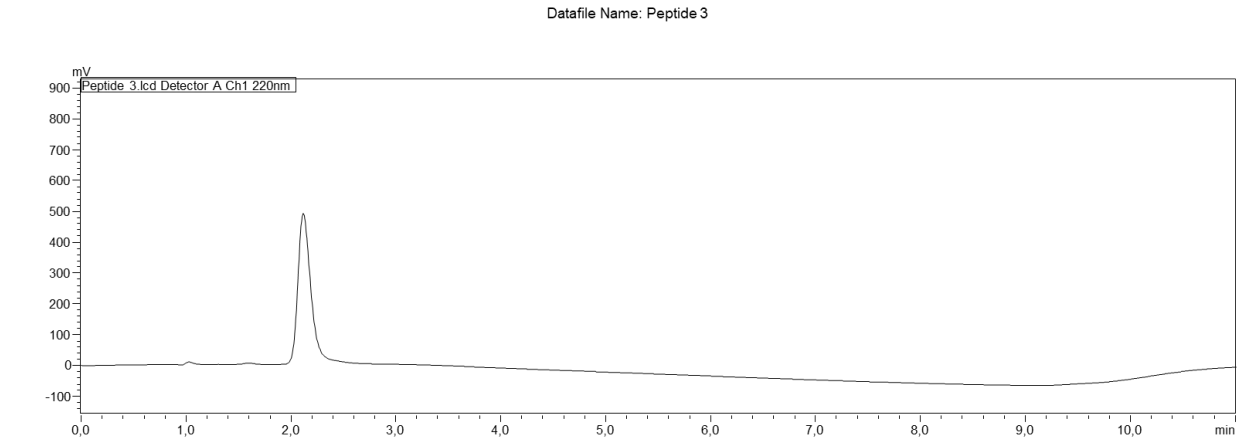


Figure S1. HR-ESI-MS of Peptide **3** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.

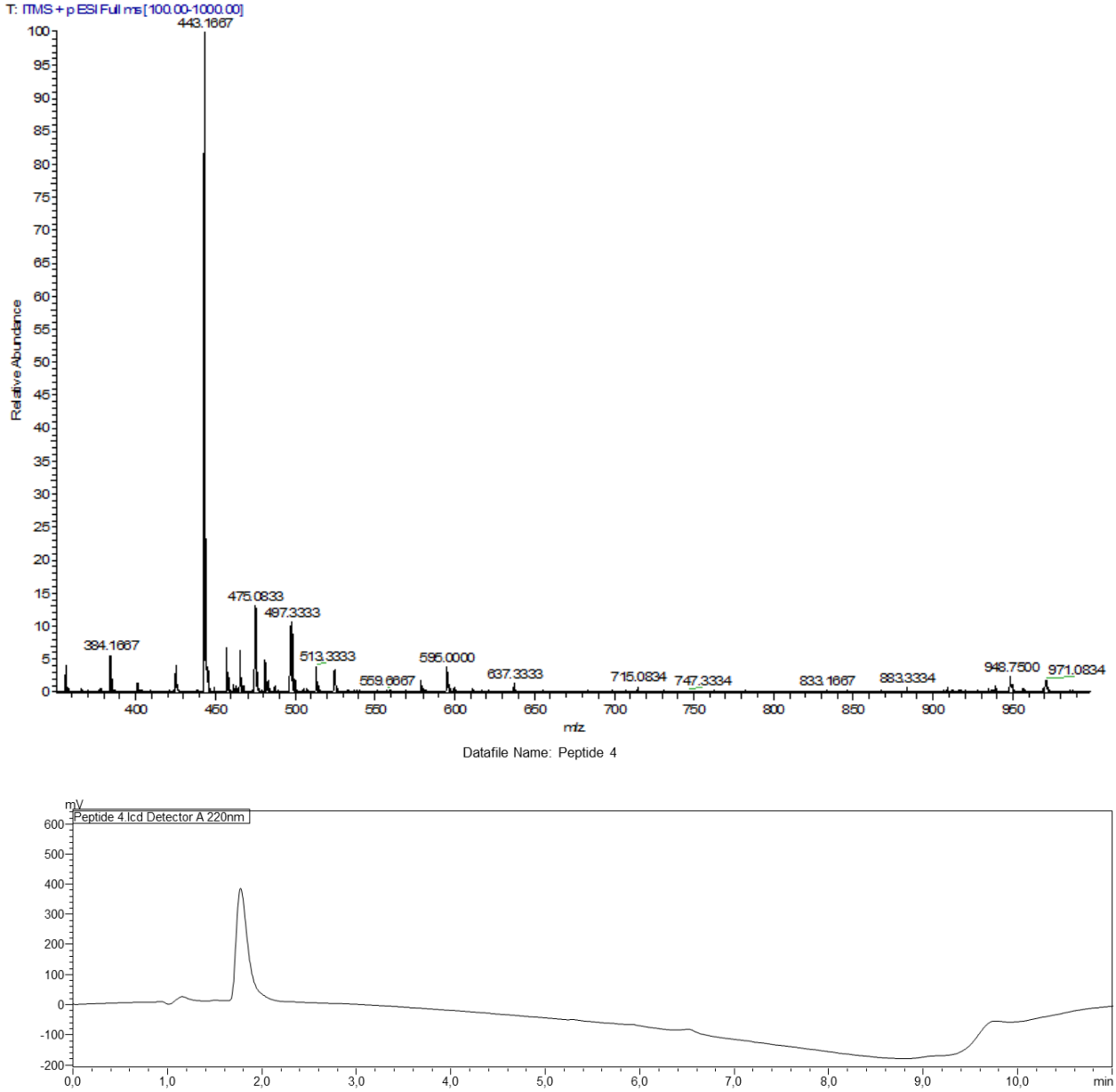


Figure S2. HR-ESI-MS of Peptide **4** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.

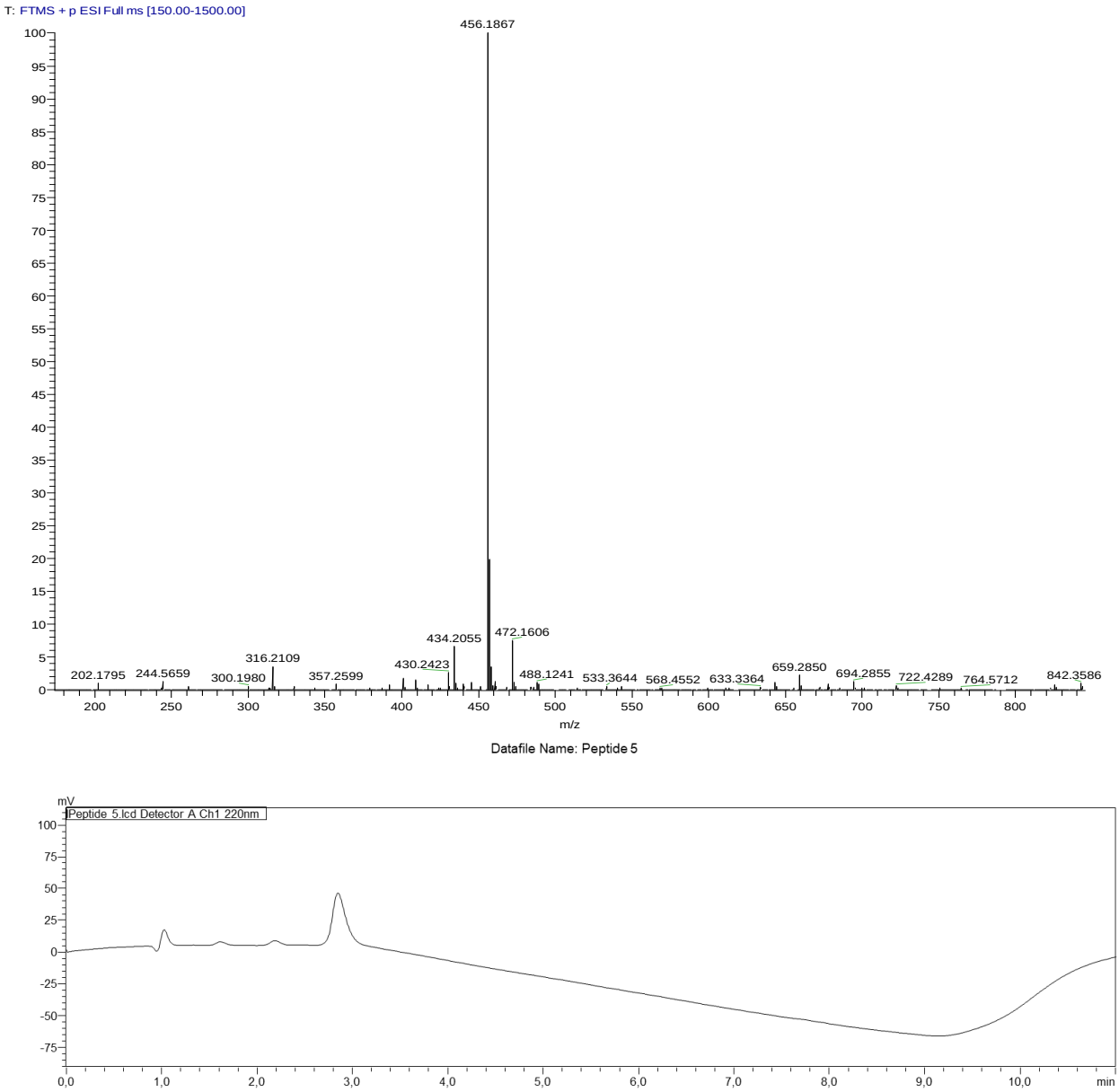
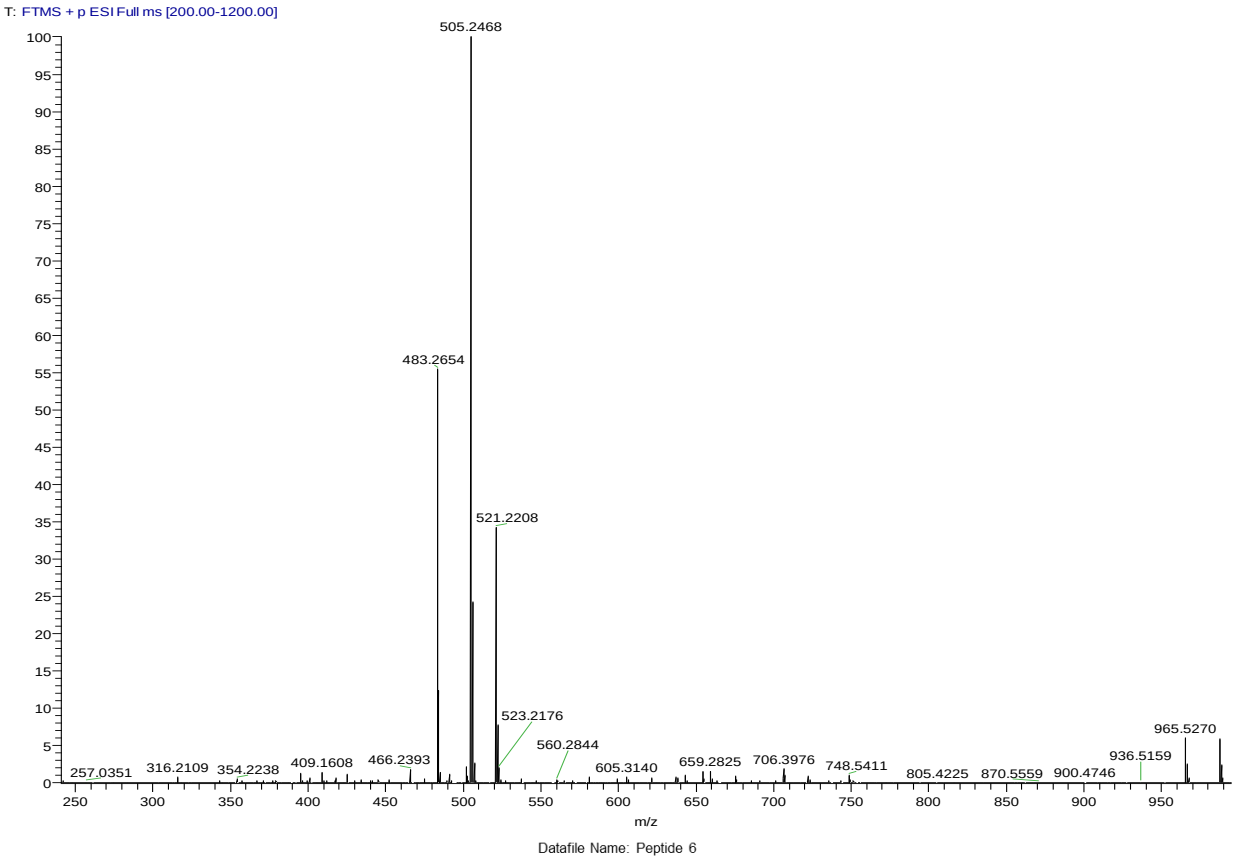


Figure S3. HR-ESI-MS of Peptide **5** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.



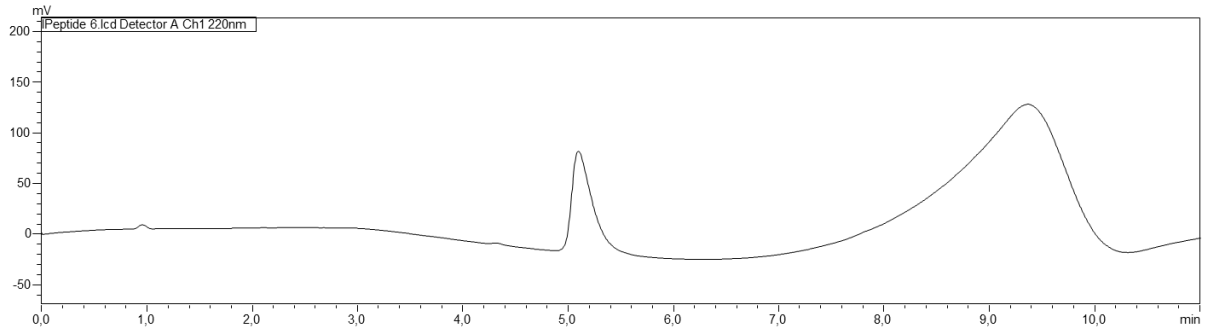
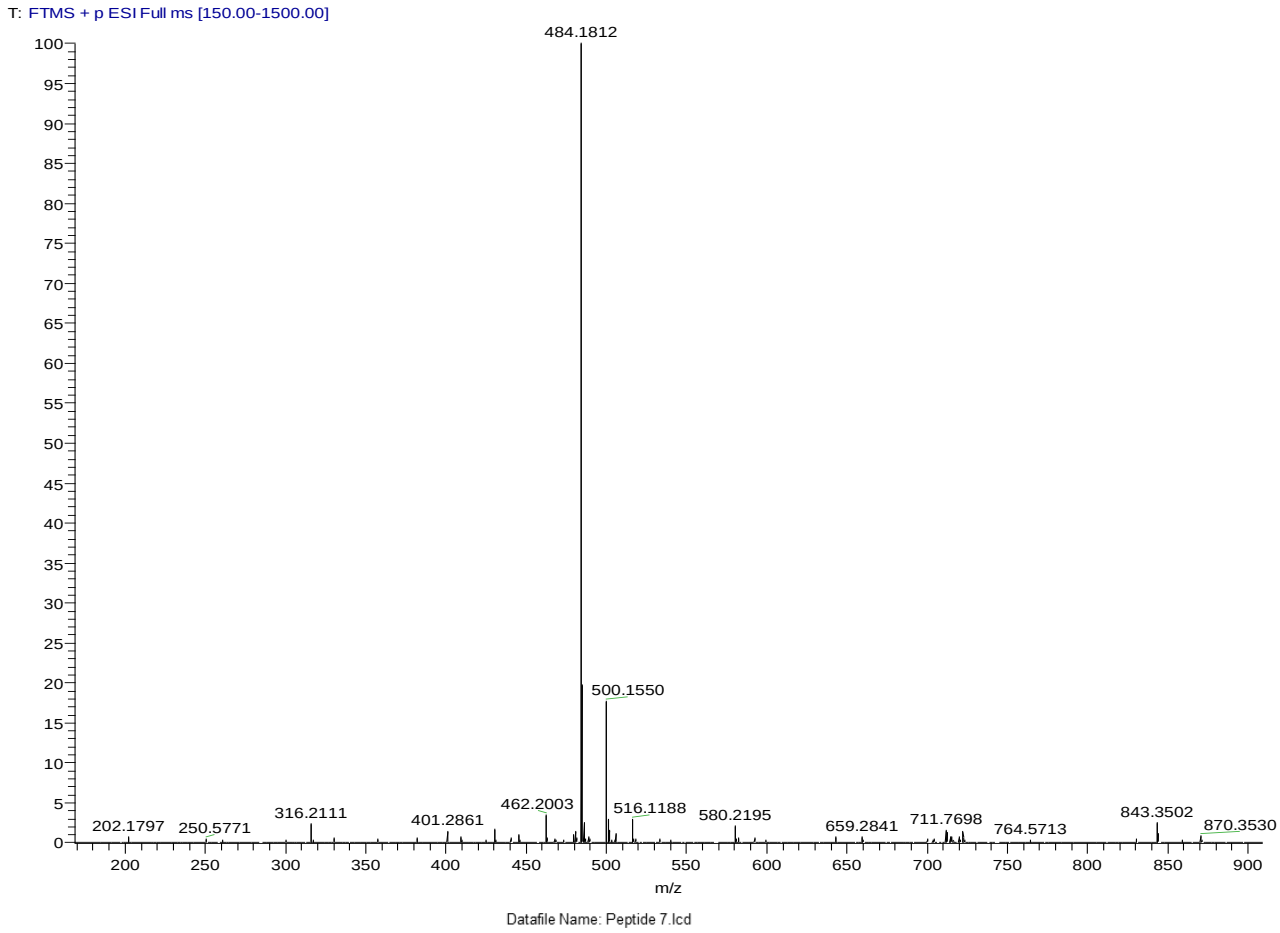


Figure S4. HR-ESI-MS of Peptide **6** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.



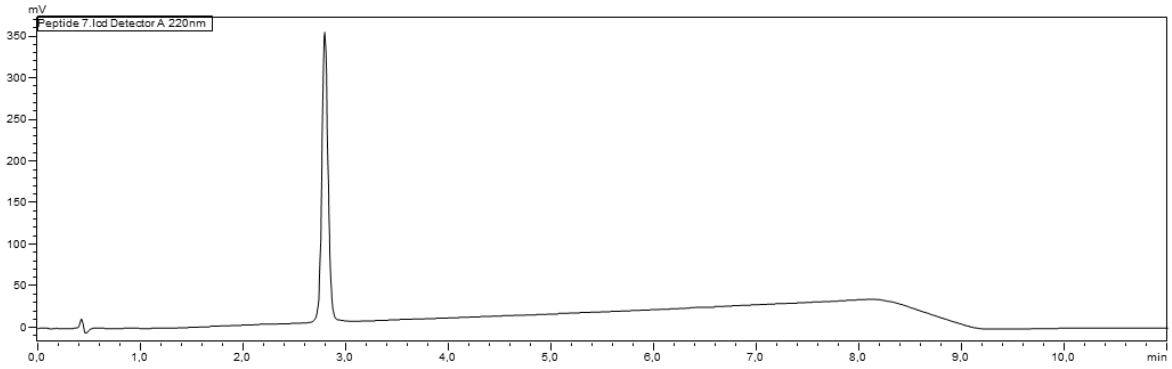
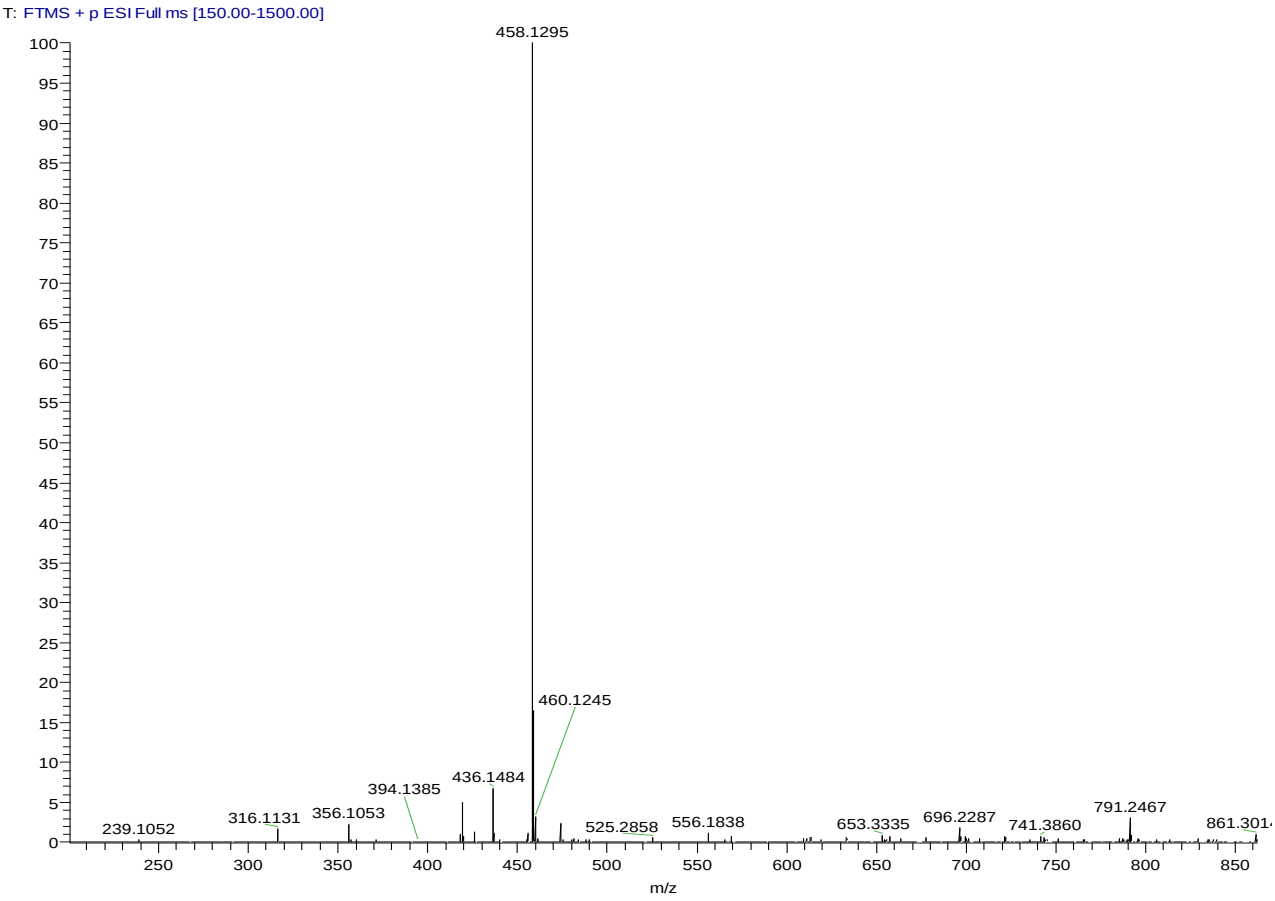


Figure S5. HR-ESI-MS of Peptide **7** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.



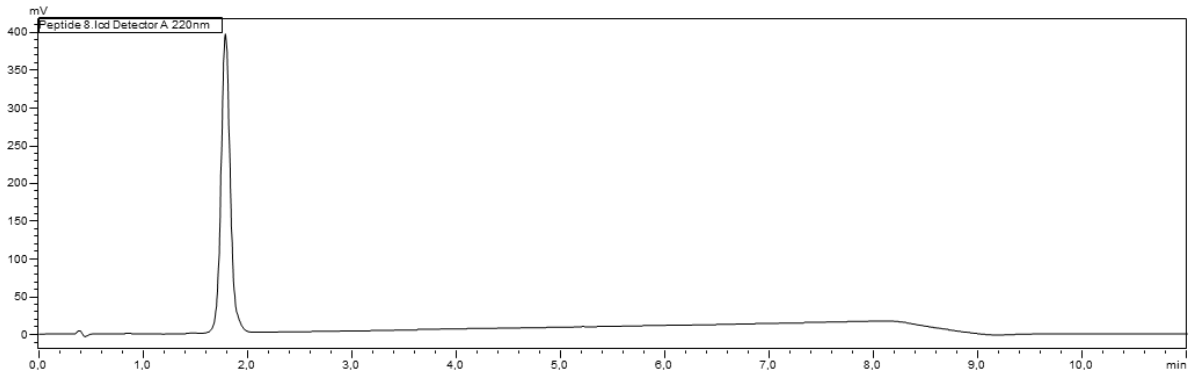


Figure S6. HR-ESI-MS of Peptide **8** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.

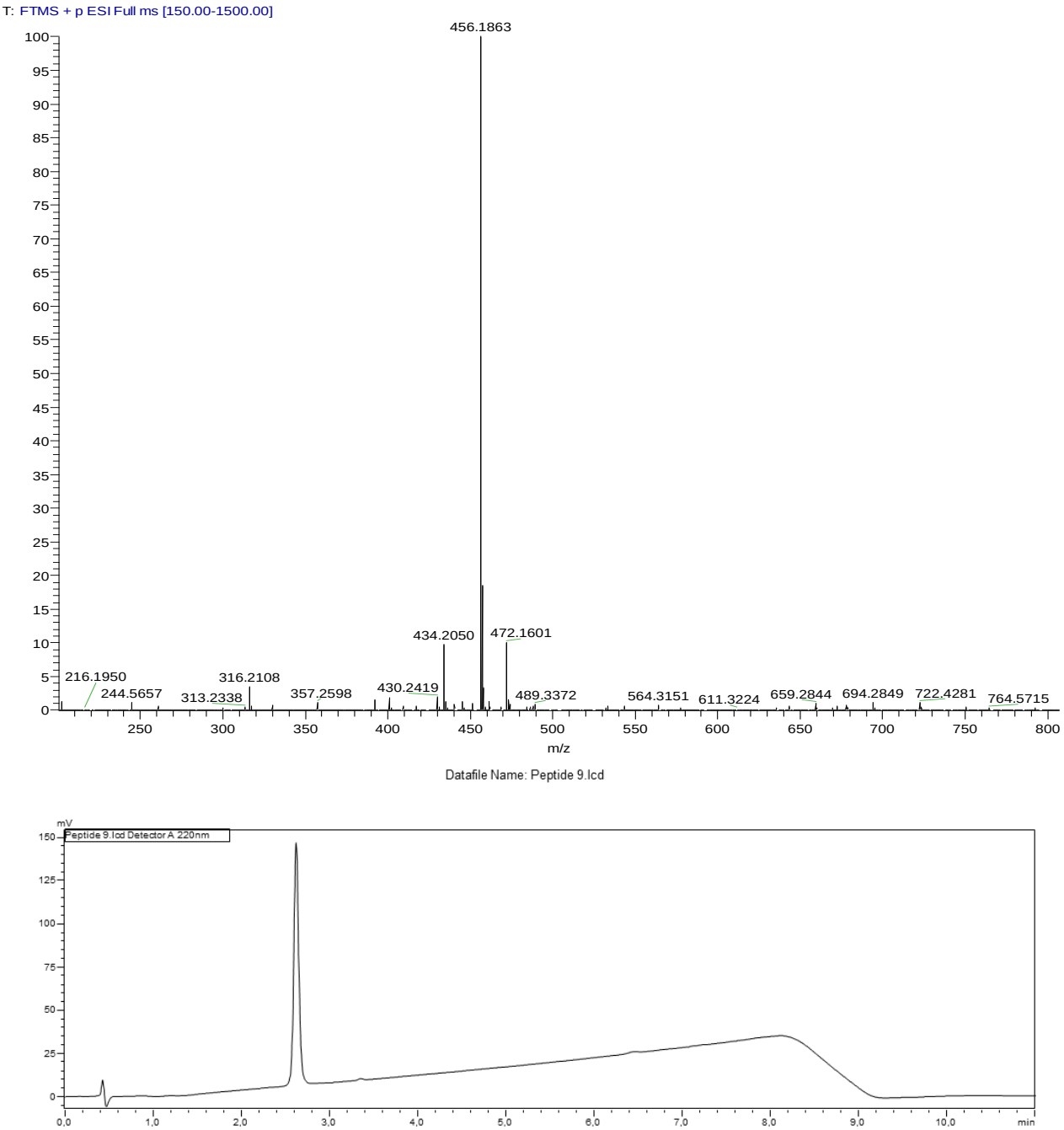
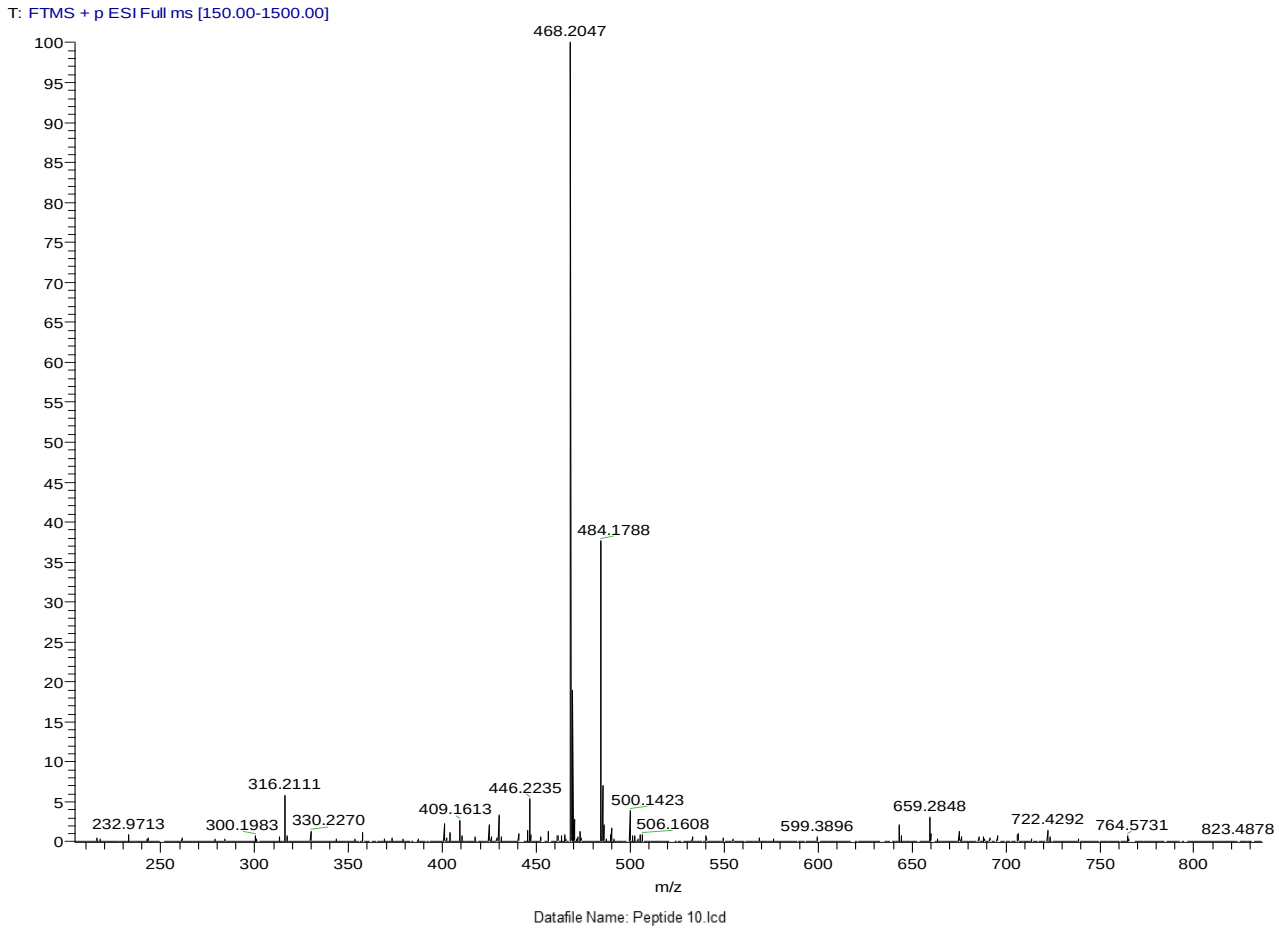


Figure S7. HR-ESI-MS of Peptide **9** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.



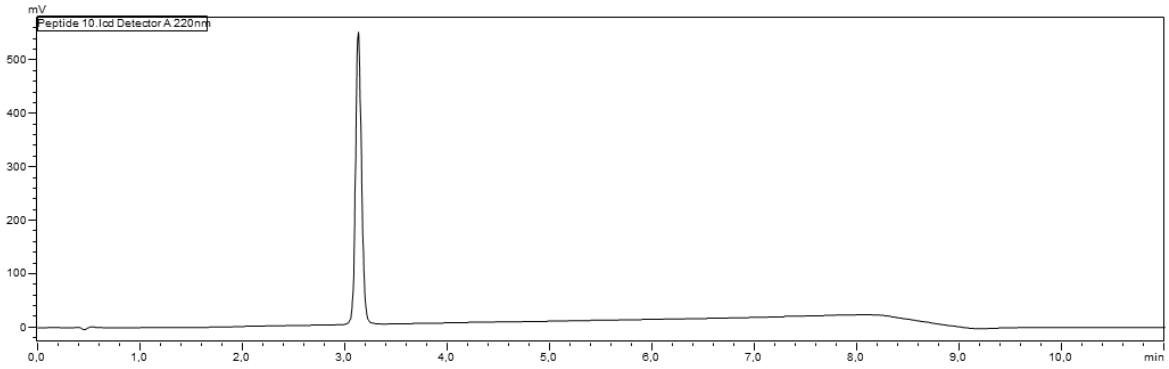


Figure S8. HR-ESI-MS of Peptide **10** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.

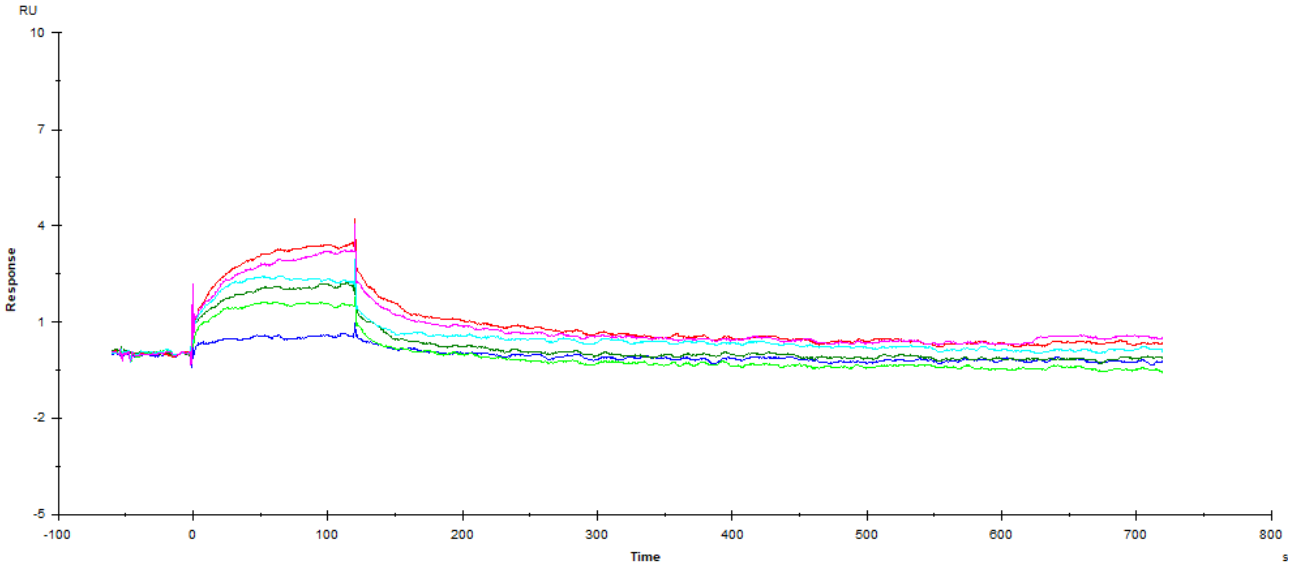


Figure S9. SPR sensorgrams between HA (immobilized on the sensor chip surface) and the compound **3**.

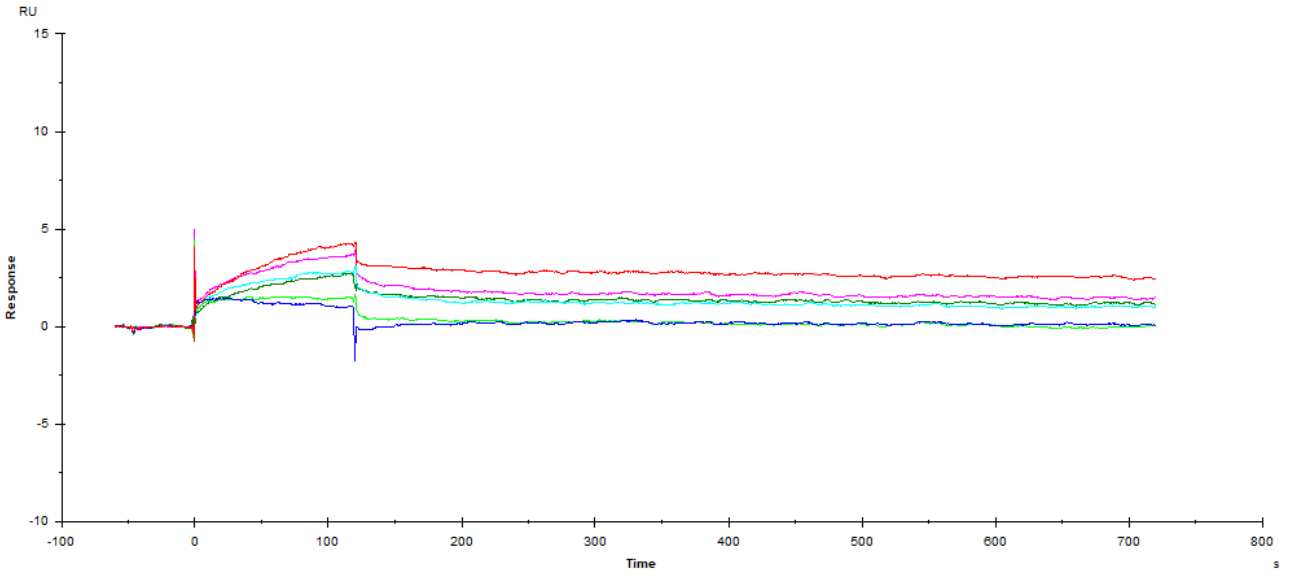


Figure S10. SPR sensorgrams between HA (immobilized on the sensor chip surface) and the compound **5**.

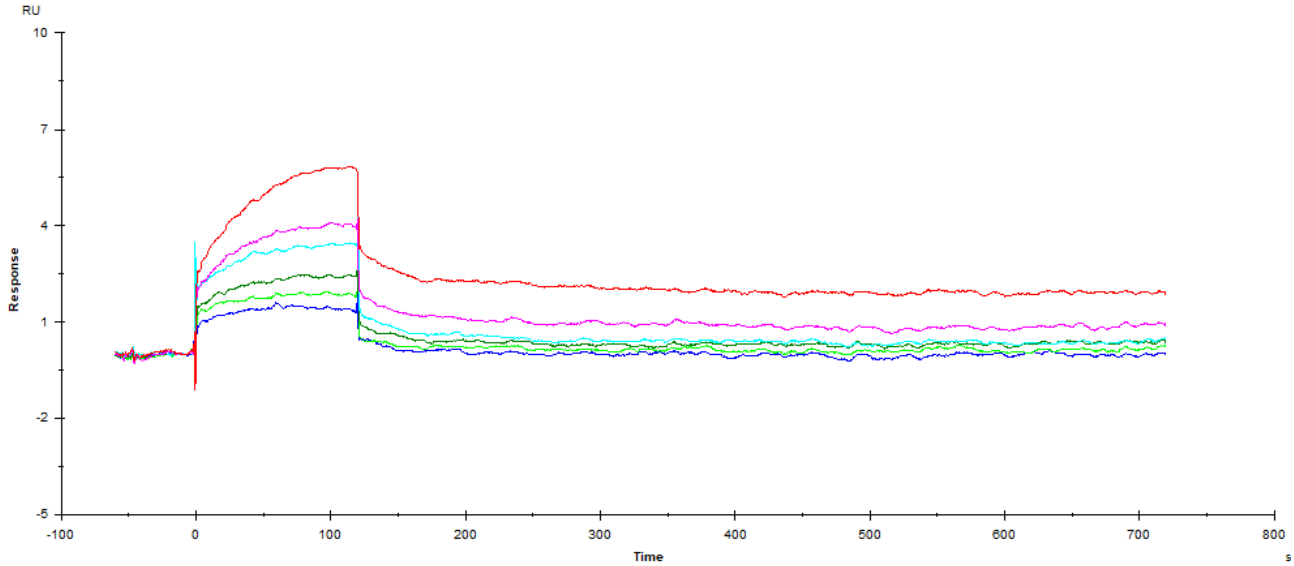


Figure S11. SPR sensorgrams between HA (immobilized on the sensor chip surface) and the compound **6**.

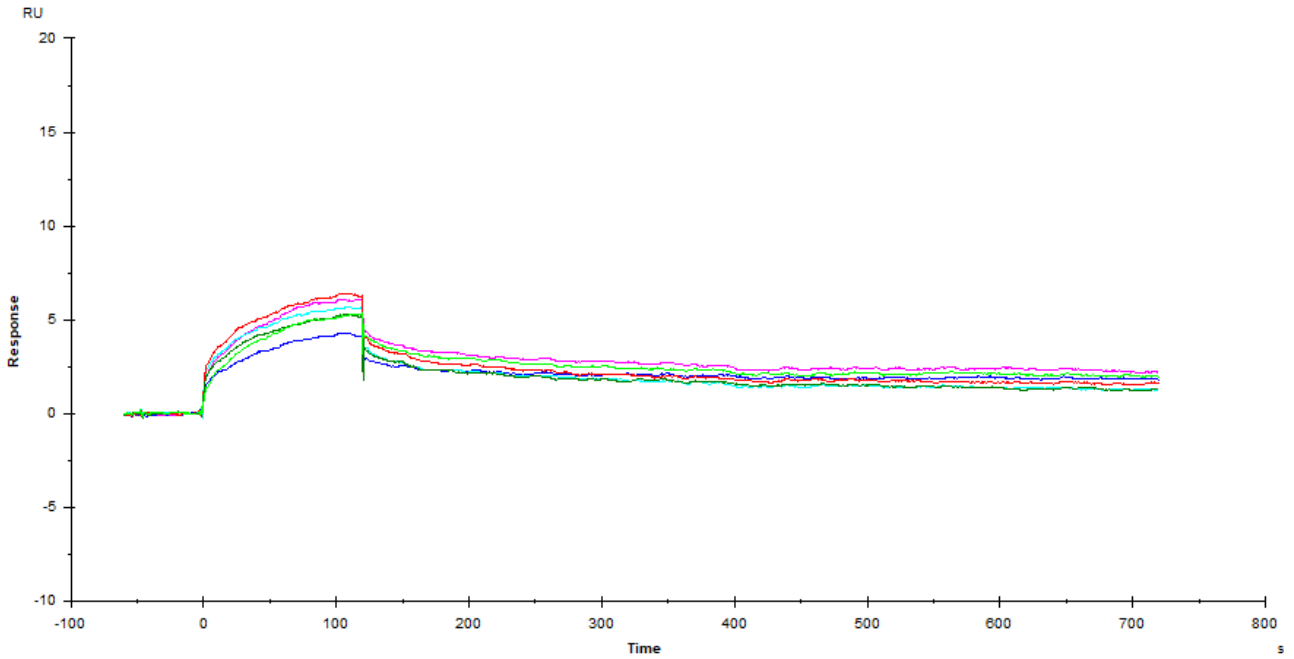


Figure S12. SPR sensorgrams between HA (immobilized on the sensor chip surface) and the compound **7**.

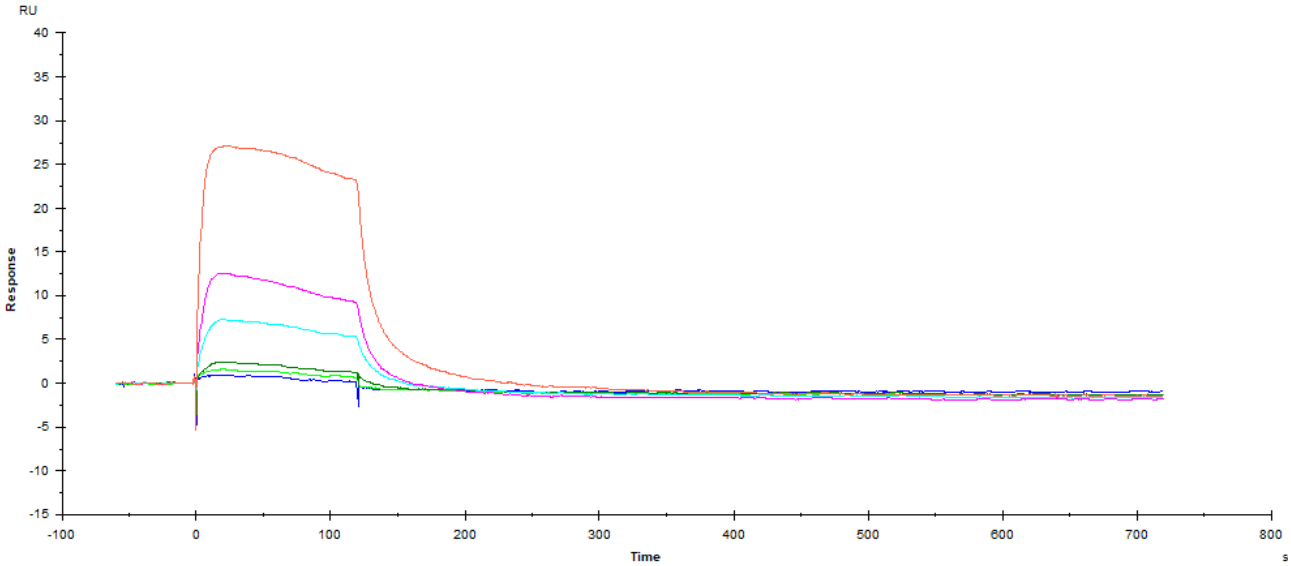


Figure S13. SPR sensorgrams between HA (immobilized on the sensor chip surface) and the compound **8**.

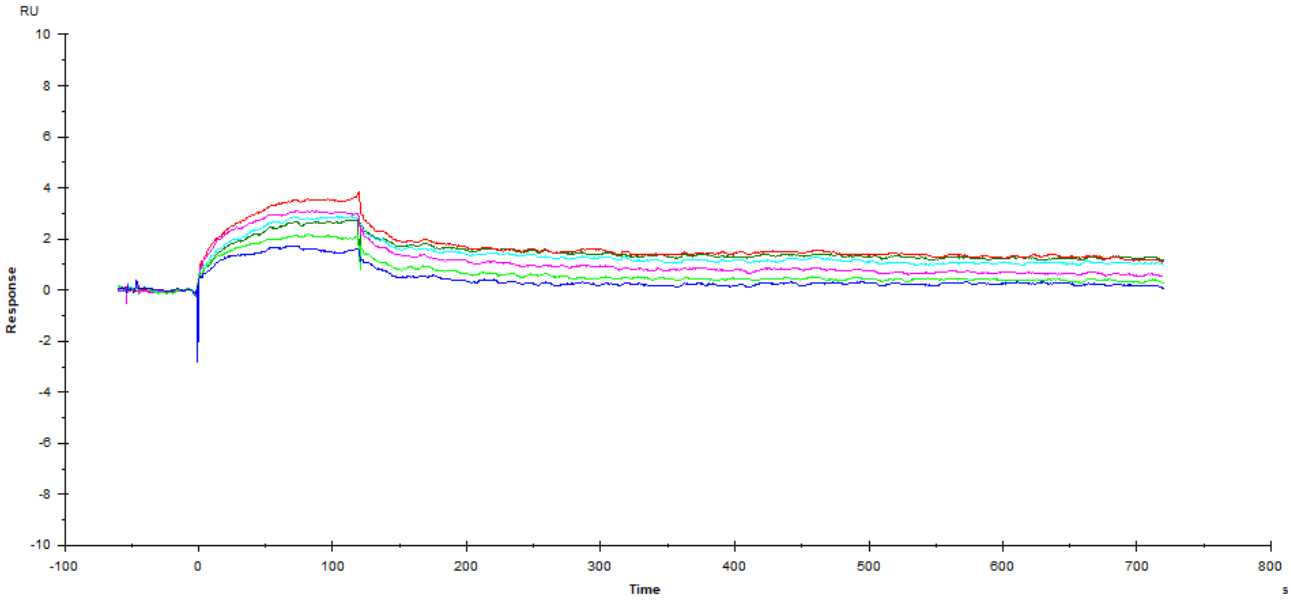


Figure S14. SPR sensorgrams between HA (immobilized on the sensor chip surface) and the compound **9**.

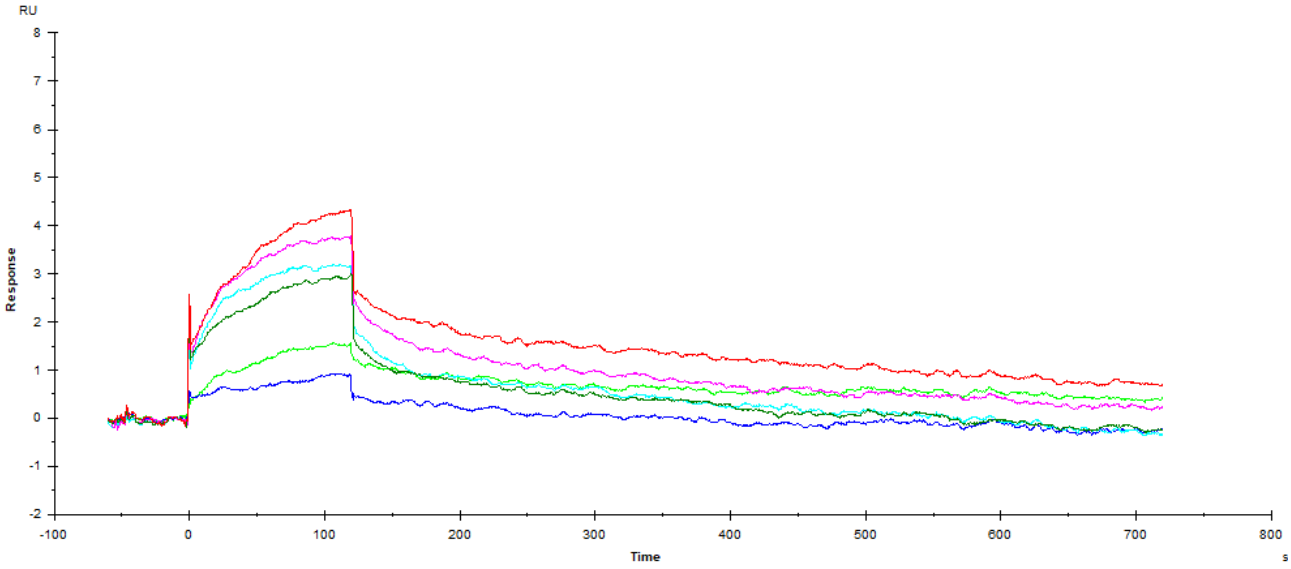


Figure S15. SPR sensorgrams between HA (immobilized on the sensor chip surface) and the compound **10**.

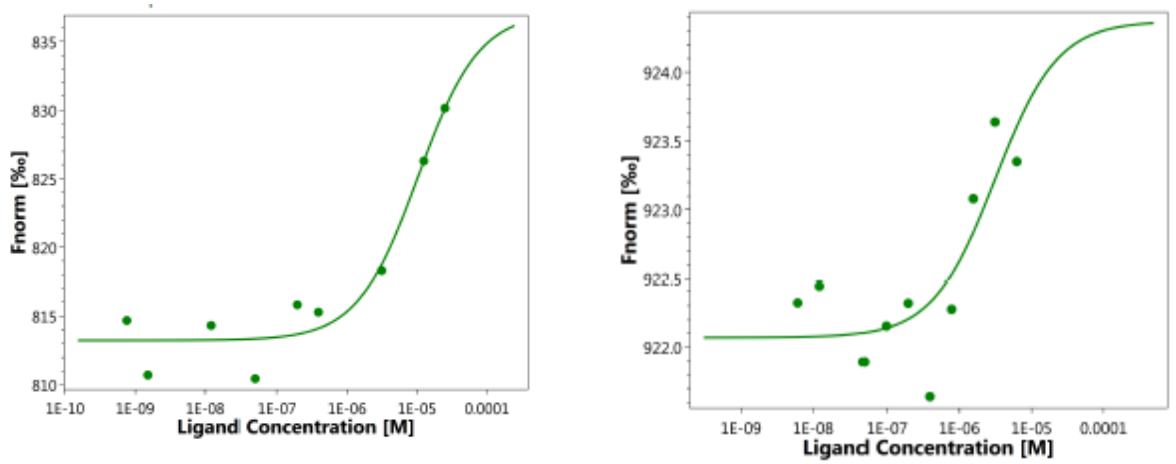


Figure S16. MST binding curve of peptide **2-3** to HA.

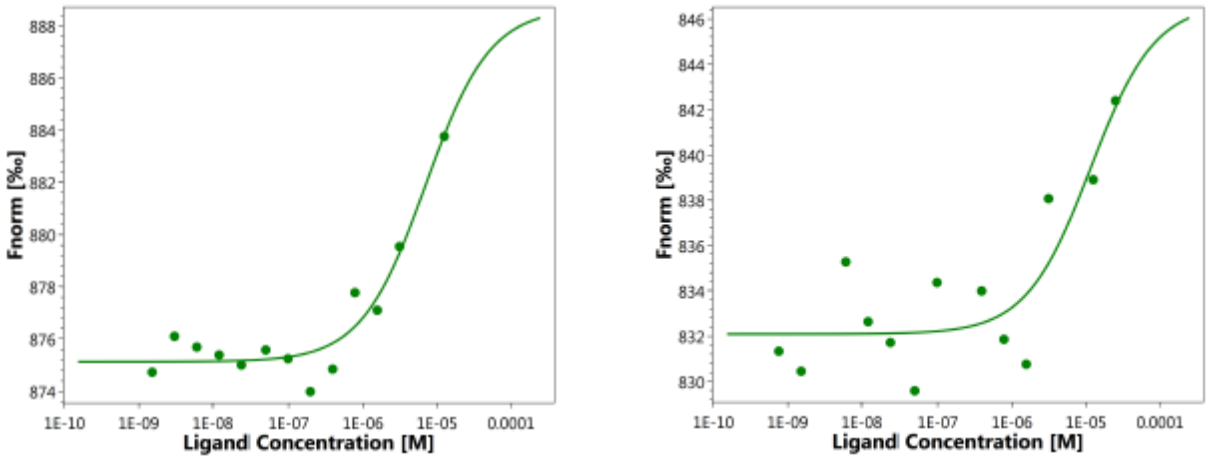


Figure S17. MST binding curve of peptide **5-6** to HA.

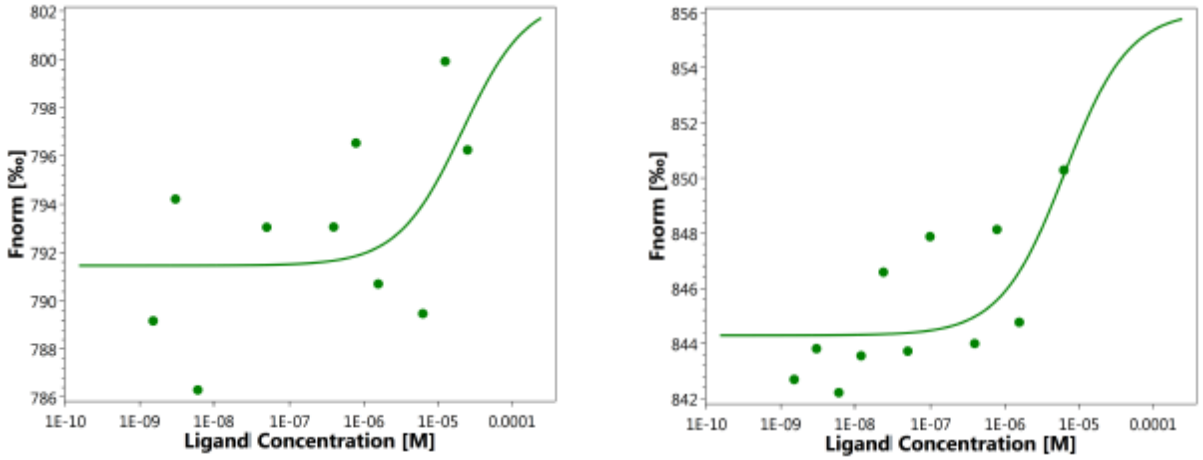


Figure S18. MST binding curve of peptide **7-8** to HA.

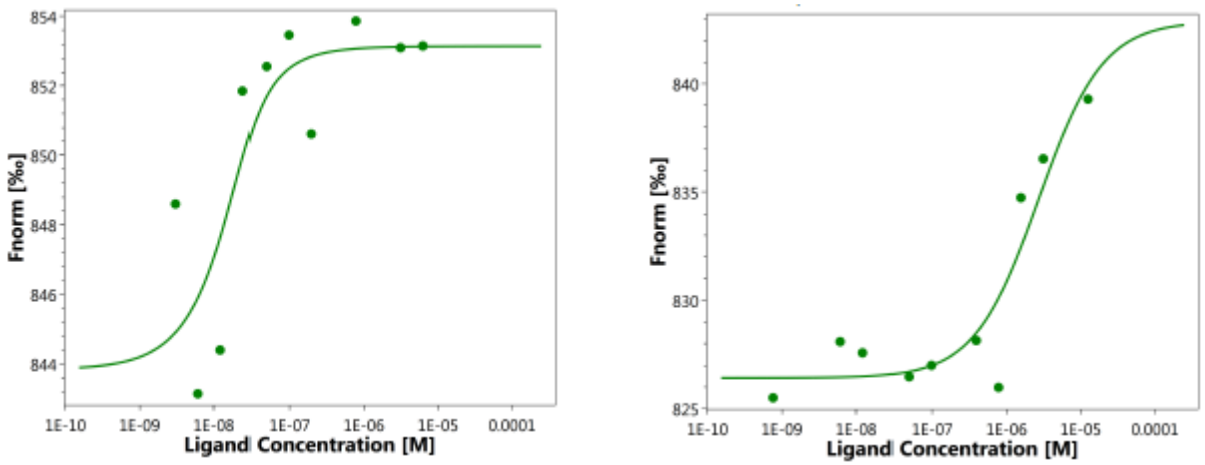


Figure S19. MST binding curve of peptide **9-10** to HA.

Table S2. Best ranking templates used for model generation in Swiss-Model for each HA

Structure	Template	Seq Identity	Oligo-state	QSQE	Found by	Method	Resolution	Seq Similarity	Coverage
Parma_H1N1	6wcr.1	85.92	hetero-3-3-mer	0.95	HHblits	X-ray	2.68Å	0.58	0.90
Roma_H1N1	6wcr.1	85.95	hetero-3-3-mer	0.96	HHblits	X-ray	2.68Å	0.58	0.90
Parma_H3N2	6aop.1	98.17	hetero-3-3-mer	0.97	HHblits	X-ray	2.30Å	0.62	0.90

Table S3. Score values obtained for the best homology models

Structure	Built with	Oligo-state	GMQE	QMEAN
Parma_H1N1	ProMod3 3.1.1	hetero-3-3-mer	0.85	-0.16
Roma_H1N1	ProMod3 3.1.1	hetero-3-3-mer	0.84	-0.08
Parma_H3N2	ProMod3 3.1.1	hetero-3-3-mer	0.90	-0.06

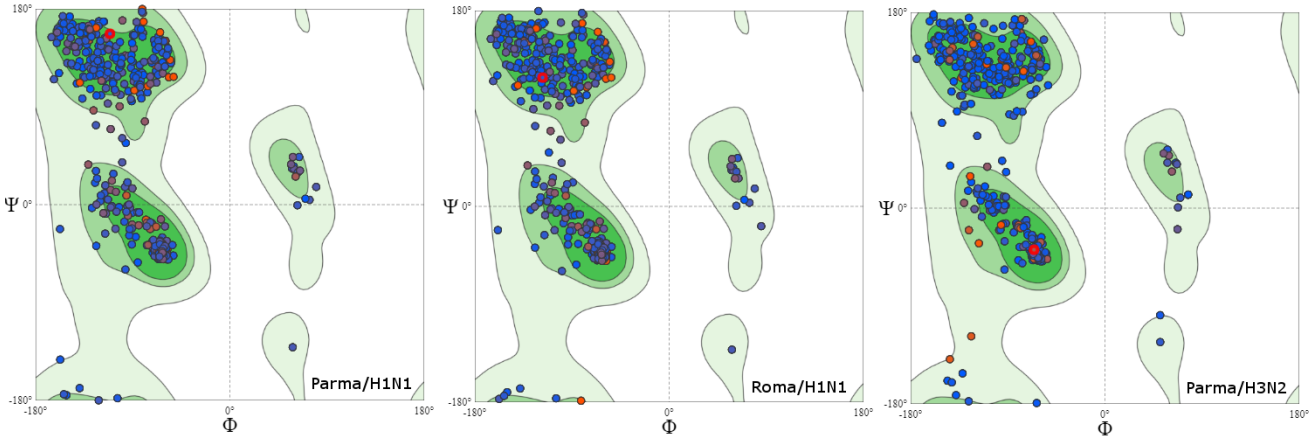


Figure S20. Ramachandran Plots obtained for HA homology models

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H1N1_Parma   1     ------STDTVDTVLEKNVTVTHSVNLLENSHNGKLCLLKGIA
H1N1_Roma    1     ANNSTDTVDTVLEKNVTVTHSVNLLENSHNGKLCLLKGIA

H1N1_Parma   38    PLQLGNCSVAGWILGNPECELLISKESWSYIVEKPNPENG
H1N1_Roma    41    PLQLGNCSVAGWILGNPECELLISKESWSYIVEKPNPENG

H1N1_Parma   78    TCYPGHFADYEELREQLSSVSSFERFEIFPKESSWPNHTV
H1N1_Roma    81    TCYPGHFADYEELREQLSSVSSFERFEIFPKESSWPNHTV

H1N1_Parma   118   TGVS--SCSHNGENSFYRNLLWLTGKNGLYPNLSKSYANNK
H1N1_Roma    121   TGVSASCSHNGESSFYRNLLWLTGKNGLYPNLSKSYANNK

H1N1_Parma   157   EKEVLVLWGVHHPPNIADQKTLYHTENAYVSVVSSHYSRK
H1N1_Roma    161   EKEVLVLWGVHHPPNIGDQKALYHTENAYVSVVSSHYSRK

H1N1_Parma   197   FTPEIAKRPKVRDQEGRINYYWTLLEPGDTIIFEANGNLI
H1N1_Roma    201   FTPEIAKRPKVRDQEGRINYYWTLLEPRDTIIFEANGNLI

H1N1_Parma   237   APRYAFALSRGFGSGIINSNAPMDKCDAKCQTPQGAINSS
H1N1_Roma    241   APRYAFALSRGFGSGIINSNAPMDKCDAKCQTPQGAINSS

H1N1_Parma   277   LPFQNVHPVTIGECPKYVRSAKLRMVTGLRNIPSGLFGAI
H1N1_Roma    281   LPFQNVHPVTIGECPKYVRSAKLRMVTGLRNIPS-----GAI

H1N1_Parma   7     AGFIEGGWTGMVDGWYGYHHQNEQGSGYAADQKSTQNAIN
H1N1_Roma    4     AGFIEGGWTGMVDGWYGYHHQNEQGSGYAADQKSTQNAIN

H1N1_Parma   47    GITNKVNSVIEKMNTQFTAVGKEFNKLERRMENLNKKVDD
H1N1_Roma    44    GITNKVNSVIEKMNTQFTAVGKEFNKLERRMENLNKKVDD

H1N1_Parma   87    GFIDIWTYNAELLVLLENERTLDFHDSNVKNLYEKVKSQL
H1N1_Roma    84    GFIDIWTYNAELLVLLENERTLDFHDSNVKNLYEKVKSQL

H1N1_Parma   127   KNNAKEIGNGCFEFYHKCNDECMESVKNGTYDYPKYSEES
H1N1_Roma    124   KNNAKEIGNGCFEFYHKCNDECMESVKNGTYDYPKYSEES

H1N1_Parma   167   KLNRE------STDTVDTVLEKNVTVTHSVNLLENSHNGKLCL
H1N1_Roma    164   KLNREANNSTDTVDTVLEKNVTVTHSVNLLENSHNGKLCL
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Figure S21. Alignment of HA sequences of A/Parma and A/Roma H1N1 strains

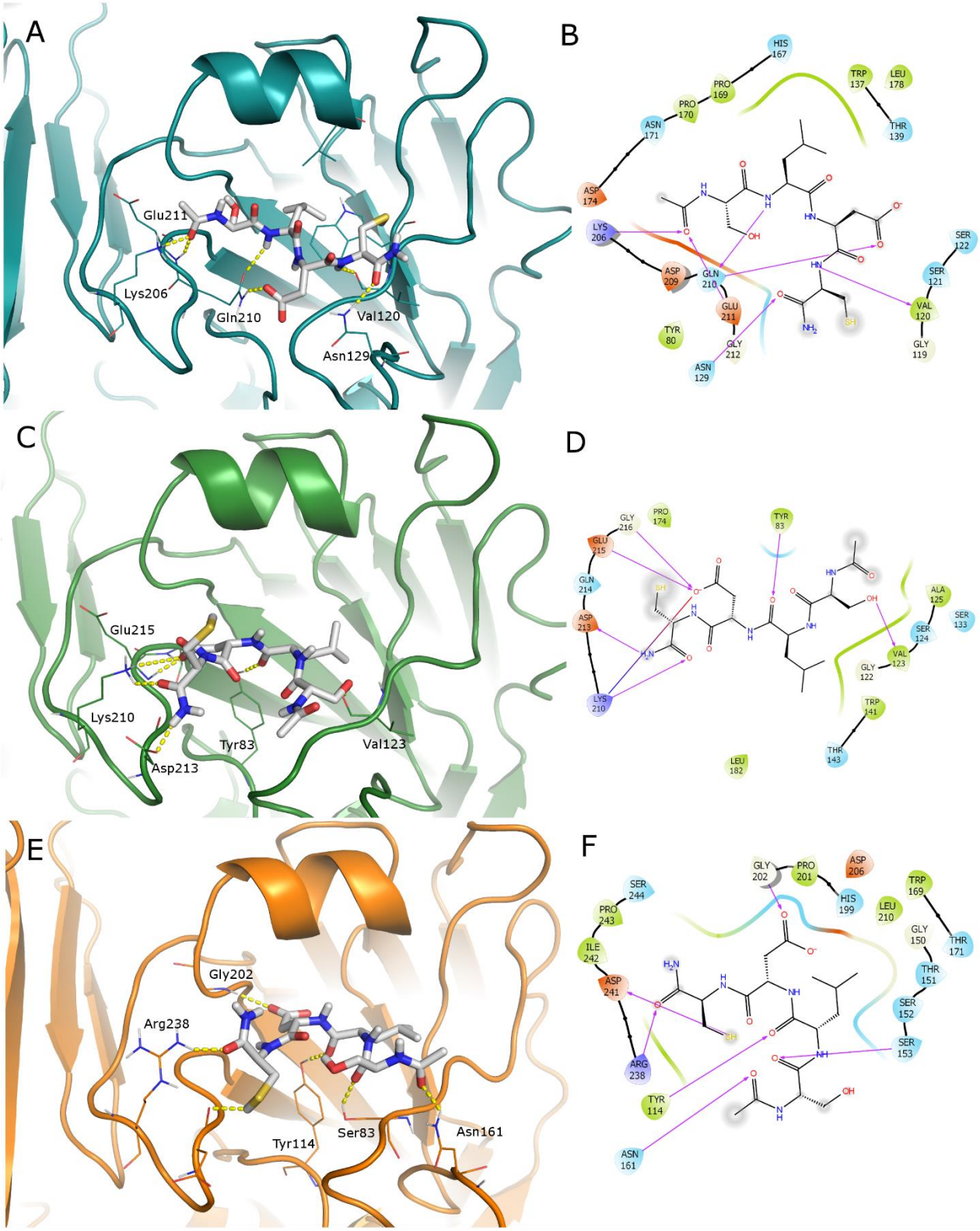

Figure S22. Docked poses of peptide **2** (white C atoms, represented as stick) in the RBS of studied HAs represented as cartoon: A) Parma/H1N1 (deep-cyan); B) Roma/H1N1 (dark green); C) Parma/H3N2 (orange). Residues involved in H-bond interactions with the ligand are represented as lines; H-bond are depicted as yellow dashed lines. Corresponding 2D ligand interaction diagrams are reported in panels B), D) and E); in the diagrams residues close to the ligand are coloured on the basis of their properties (orange, negatively charged; blue, positively charged; green, hydrophobic; cyan, polar), H-bonds are depicted as magenta arrows, solvent exposed atoms are surrounded by a grey shadow.

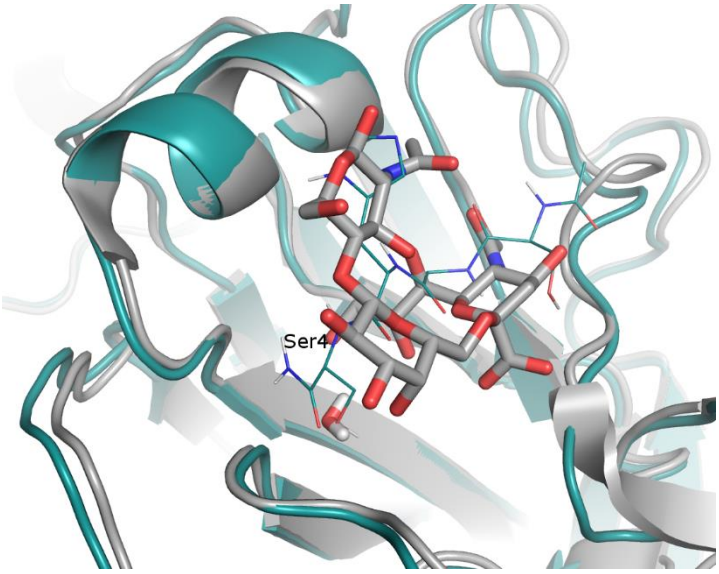

Figure S23. Superposition of the complex of HA:sialic acid (PDB ID 3UBN, grey carbon atoms) with docked pose of SAHS in the A/Parma/24/09 H1N1 (deepcyan carbon atoms). Proteins are represented as cartoon. Sialic acid and conserved water molecule are represented as stick, while the SAHS is represented as thin stick.