Influenza A H1 & H3 Transmembrane Domains Interact Differently with Each Other & with Surrounding Membrane Lipids

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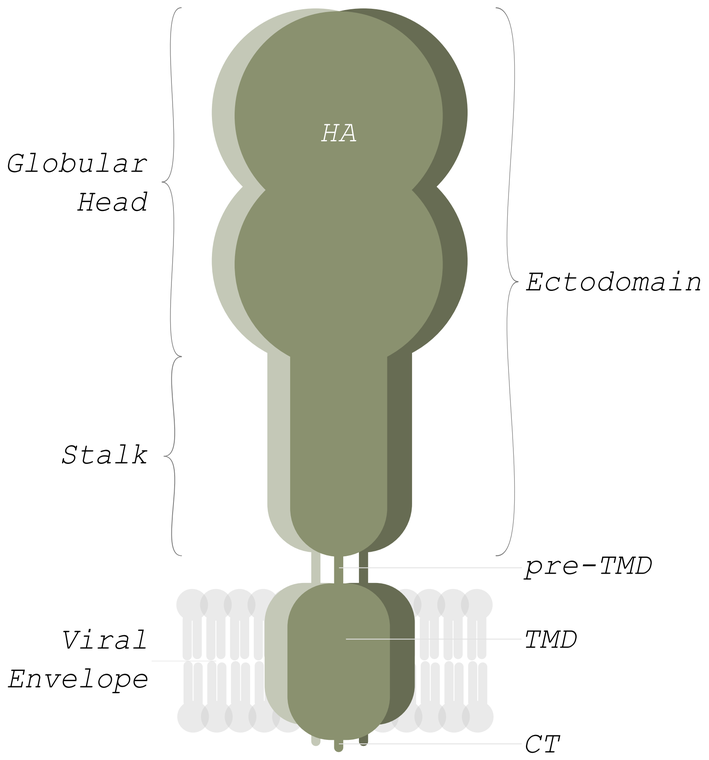
Background

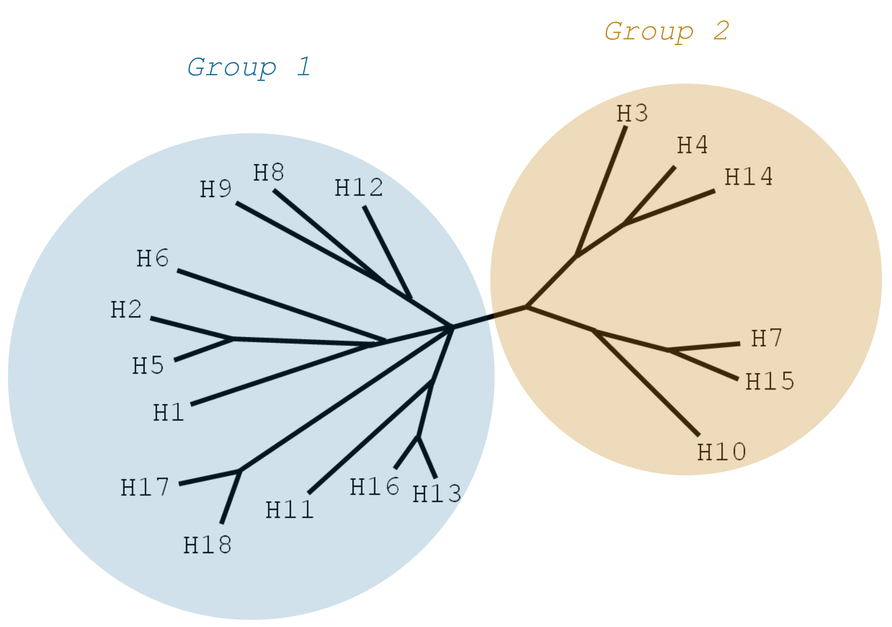
Influenza A virus (IAV) hemagglutinin (HA) is a key envelope glycoprotein, which plays a crucial role in the recognition of the host cell, fusion with the host cell membrane & is the major antigen in the immune response during the infection.



HA organizes in homotrimers consisting of a globular head & a conserved stalk region. HA monomers contain a hydrophilic ectodomain, a pre-transmembrane region (pre-TMD)[1], a hydrophobic transmembrane domain (TMD) & a cytoplasmic tail (CT).

The more exposed globular head is the primary target of neutralizing antibodies. The less exposed stalk is highly conserved[2] & is therefore of interest as target in the development of the universal influenza vaccine.

Often overlooked, TMD has been shown to have a key role in HA function. Particularly the H3 TMD, which has been shown to induce an increased, heterosubtypic immune response, when substituted for native HA TMD[3,4,5].

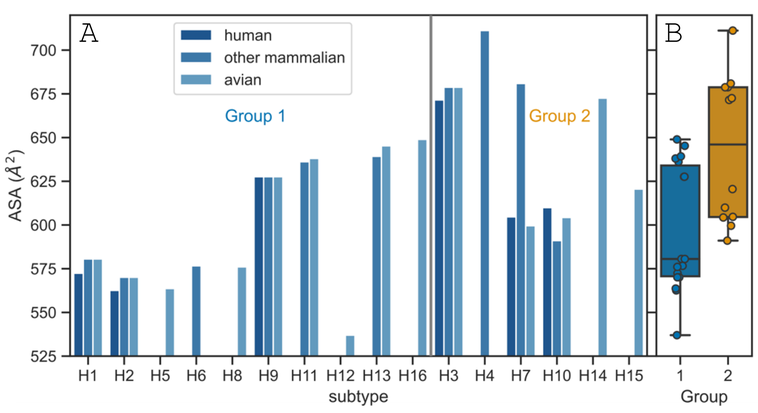


Phylogenetic analysis of 18 subtypes revealed that HA proteins can be divided into two major phylogenetic groups[6].

Methods & Results

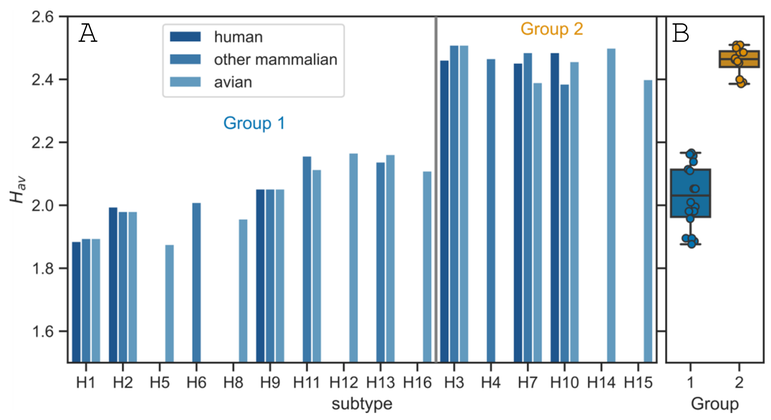
Using custom-written Python 3.7 scripts we have analyzed available amino acid sequences of 16 HA subtypes across various host species (OpenFlu DB) & calculated several physico-chemical parameters of HA TMDs & linker regions.

TMD Available Surface Area (ASA)



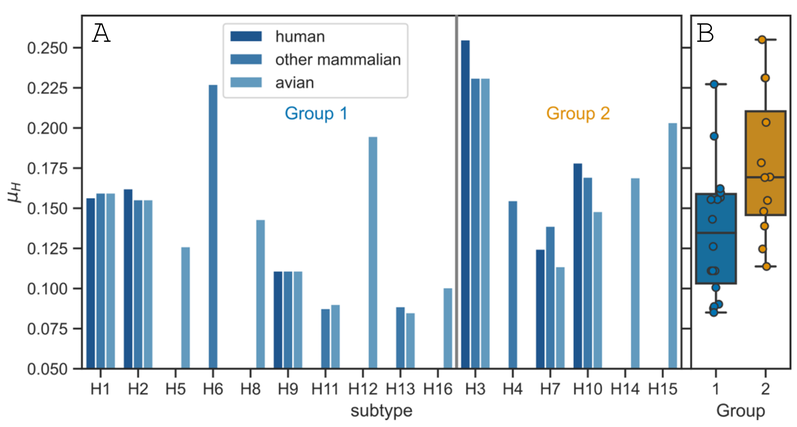
ASA of H1 TMD (577.9 ± 4.7 Å^2) was significantly lower than of H3 TMD (676.3 ± 4.2 Å^2) (A). This observation held for averaged ASA in phylogenetic Group 1 (599 ± 36 Å^2) & Group 2 (544 ± 42 Å^2) (B), $p < 0.01$, two-sided Mann-Whitney U test)

TMD Average Hydrophobicity (H_{av})



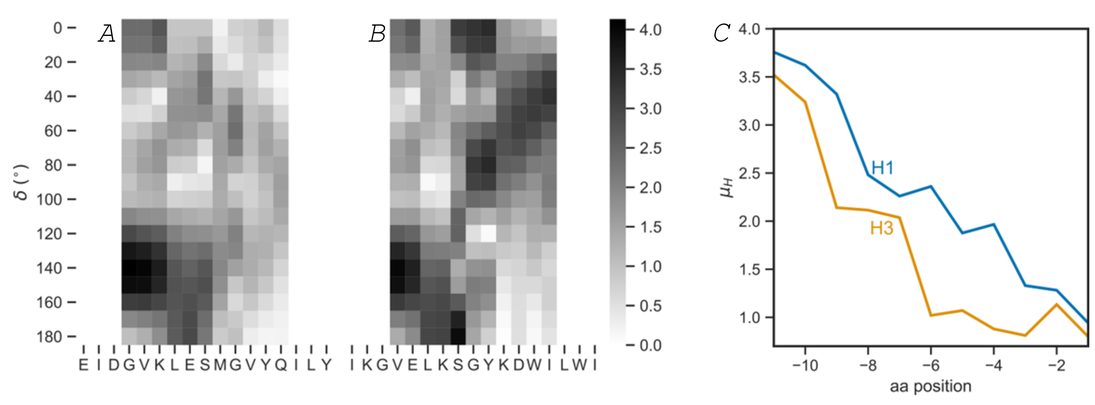
Highest TMD hydrophobicity (H_{av}) was observed for HA subtypes in Group 2 (A). On average H_{av} in Group 1 = 2.03 ± 0.1 & was significantly lower than in Group 2 (2.46 ± 0.04) (B), $p < 10^{-5}$, two-sided Mann-Whitney U test.

TMD Average Hydrophobic Moment (μ_H)



The highest amphiphilicity (measured as μ_H) was observed for H3 TMD (0.239 ± 0.014) (A). On average μH in Group 1 = 0.18 ± 0.05 & was significantly higher than in Group 2 (0.13 ± 0.04) (B), $p < 10^{-5}$, two-sided Mann-Whitney U test. Turn angle, δ = 100°

H1 & H3 pre-TMD Average Hydrophobic Moment (μ_H)



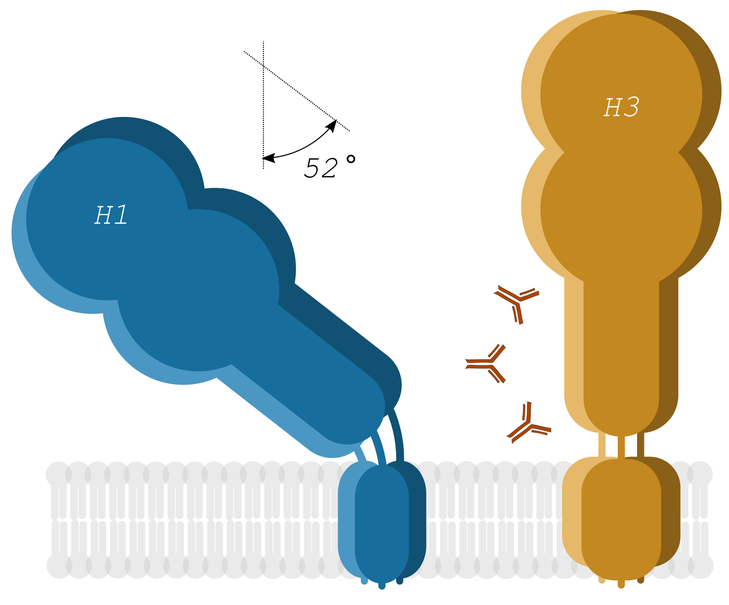
μ_H for human H1 & H3 pre-TMDs was calculated for 11 amino acid (aa) sequences upstream of their TMDs. Since HA pre-TMD is not an α-helix, we extended the calculations to δ range from 0° to 180° & created μ_H maps (A & B). μ_H map cross-section was plotted for the maximal μH at δ=140°. We observed higher μ_H values for H1 when compared to H3.

Conclusions & Hypothetical Model

We hypothesize that due to significant differences in physico-chemical properties, the interactions of pre-TMDs & TMDs with surrounding membrane lead to different positioning of H1 & H3 ectodomains

Unlike previously described H1 pre-TMD, which tilts the ectodomain at 52°[1], the H3 ectodomain could be positioned in a way that exposes the conserved epitopes of the stalk region.

This would explain the increased immunogenic & heterosubtypic effect observed in H3 TMD-containing recombinant HAs[3,4,5]



References
[1]*Benton et al., 2018;* [2]*Kirkpatrick et al., 2018;* [3]*Liu et al., 2014;* [4]*Zhang et al., 2017;* [5]*Wang et al., 2017;* [6]*Zhang et al., 2019.*

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