Interpretation of mutations (FluSurver)

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DTG

GISAID

Bioinformatics Institute

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GISAID

From the **sequence** and **structure** we can partially deduce important **properties** of the virus

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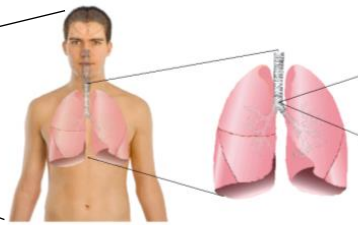
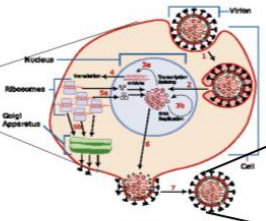
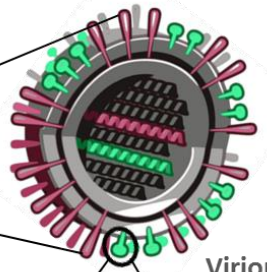
- Infect different hosts
- More or less severe
- Spread more easily
- Antigenic drift, drug resistance

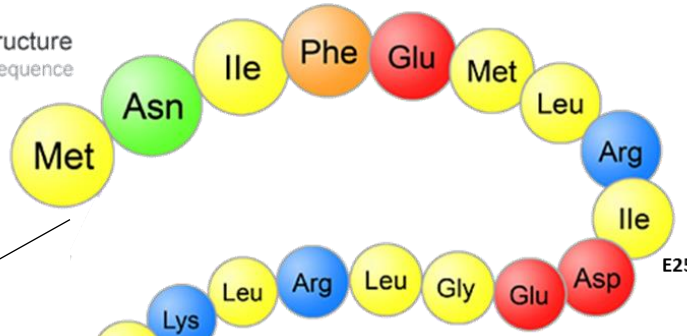




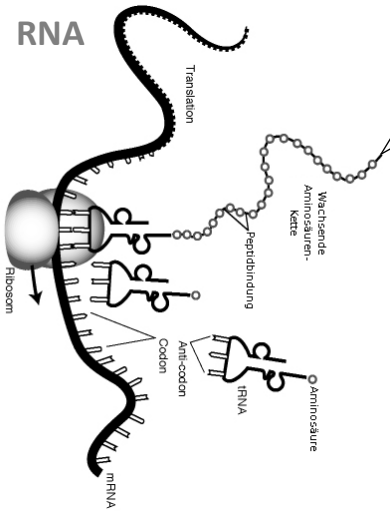


Virion
Viral Particle

Primary Structure
Amino Acid Sequence



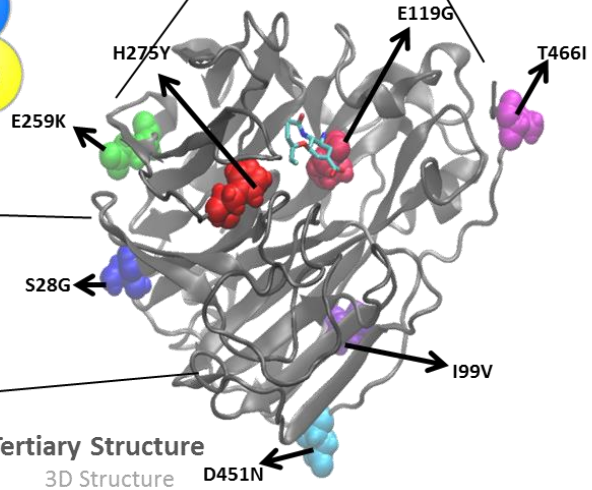
RNA



Secondary Structure
Regular Sub-Structures

α-helix

β-sheet



Protein Tertiary Structure
3D Structure

Simply paste/upload your sequence(s):

http://flusurver.bii.a-star.edu.sg

…or analysis menu from GISAID's EpiFlu

Get list of identified mutations

FluSurver

Result for comparison with reference selection: **H1N1_Human_2009_California07** Back to Reference Selection

Query	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
HA_H1N1_Human_2009_Norway3206-3 gi|269978854|gb|ACZ56080.1|	HA A/California/07/2009(H1N1) find closest related sequences	99.117	100.000	5	P100S, P154S, S220T, D239G, I338V show in structure

Get exhaustive annotation for each mutation

Quick summary

Geographic

Map of cities with the HA D239G mutation

Map of countries with the HA D239G mutation

Number of occurences

Countries without data

Alternative numberings

HA D239G

Key to alternative position numbering:

	FluSurver numbering (absolute as in 2009 H1N1 pandemic)
239	
HA1 225	Classical H3N2 strain numbering
HA1 222	Classical H1N1 strain numbering
Chosen reference:	
Position in reference:	
AA in reference:	D
AA in query:	

Mutation HA D239G already occurred 194 times (1.37% of all samples with HA sequence) in 28 countries. The first strain with this mutation, collected in April 2009, was A/Texas/11/2009(H1N1). The mutation most recently occurred in strain A/New York/09/2014(H1N1), collected in April 2014. (see map)

See detailed global statistics for this position

A mutation at the position equivalent to **HA 239** has been reported in the literature to be related to host specificity shift.

A combination of mutations including the position equivalent to **HA 239** has been reported in the literature to be related to host specificity shift.

As seen in resolved structures of proteins from related strains, the HA position equivalent to your mutation is involved in:
- host cell receptor binding
- viral oligomerization interfaces
- binding small ligand(s)
- antibody recognition sites

See all interactions for this position

PubMed search for this mutation (including alternative numbering)

Structure models

Reference: HA_H1N1_Human_2009_California07 (Structure/Model Details)
Patient/Sample: HA_H1N1_Human_2009_Norway3206-3 gi|269978854|gb|ACZ56080.1|
Mutation(s): *I338V, S220T, P100S, P154S, D239G*
Wildtype residue only displayed if position is part of available structure.
Hover with mouse over residue to see its position number. Right-click for more options.

Temporal

Frequency of mutation over time

100 / 75 / 50 / 25 / 0

2009 2010 2011 2012 2013

Structural interactions

Effect with Literature links

Known effect(s) of mutations at position equivalent to your mutation:

Protein: HA
Influenza type: Avian, Human H1N1 (2009)
Mutation (as in paper): D222G or D225G
neutral AA: D
neg. eff. AA: G
Effect: host specificity shift

Comment:

HA D239G is also referred to in the literature as D222G or D225G using alternative (e.g. seasonal H1/H3) numberings. It has been found to alter host cell receptor specificity from human alpha-2,6 to also include avian-like alpha-2,3 [illegible] which are common in ciliated human cells of the lower respiratory tract. While this mutation has been found in higher proportions in severe cases, it is IMPORTANT to note that it also can occur as a [illegible] its effect would only be relevant for surveillance if the mutation is also found in the original clinical sample.

Literature reference

(Mutation D222G or D225G in the paper is at an equivalent position of the mutation in your query)

Literature-curated genotype to phenotype effect annotations

Effect Type	# Annotations
host specificity shift	136
virulence	106
antigenic drift / escape mutant	84
strong drug sensitivity change	40
mild drug sensitivity change	30
other	23
total (2015)	**419**

Example:

Known effect(s) of mutations at position equivalent to your mutation:

Protein: HA
Influenza type: Avian, Human H1N1 (2009)
Mutation (as in paper): D222G or D225G
neutral AA: D
neg. eff. AA: G
Effect: host specificity shift

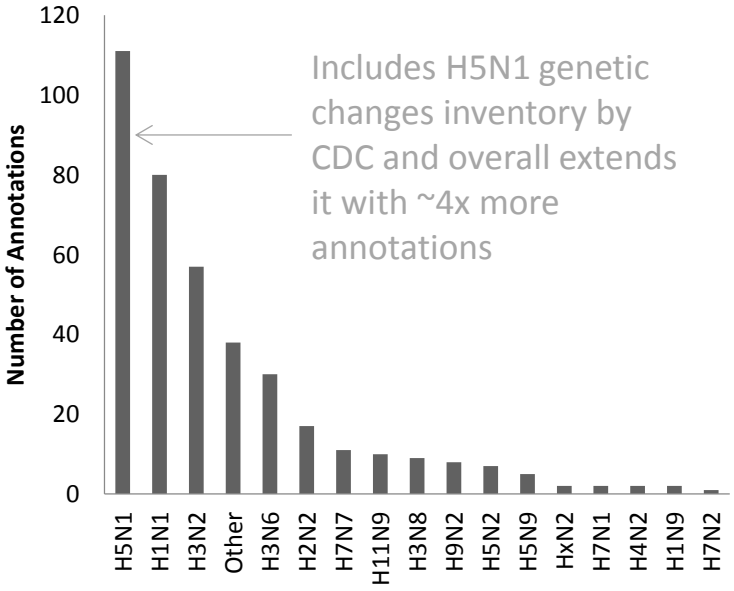
Comment:

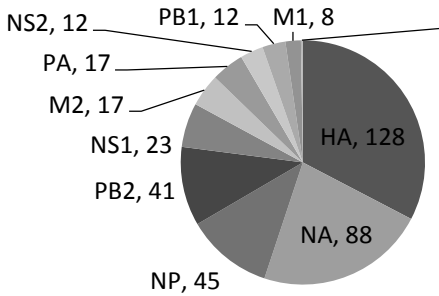
HA D239G is also referred to in the literature as D222G or D225G using alternative (e.g. seasonal H1/H3) numberings. It has been found to alter host cell receptor specificity from human alpha-2,6 to also include avian-like alpha-2,3 sialic acid which is more common in ciliated human cells of the lower respiratory tract. While this mutation has been found in higher proportions in severe cases, it is IMPORTANT to note that it also can occur as egg or cell culture adaptation. Therefore, its effect would only be relevant for surveillance if the mutation is also found in the original clinical sample.

Literature reference

(Mutation D222G or D225G in the paper is at an equivalent position of the mutation in your query)

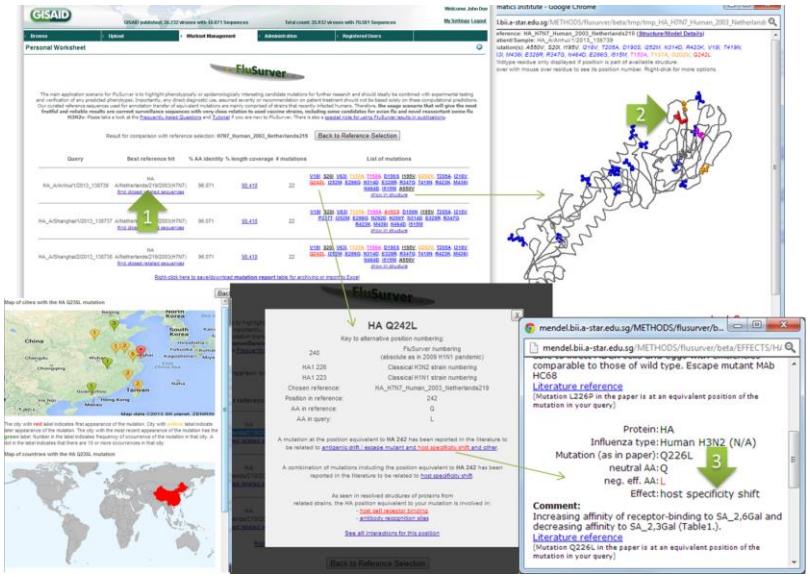
Annotations per Subtype





Annotations per Protein Type

Important – FluSurver for Mutation Interpretation

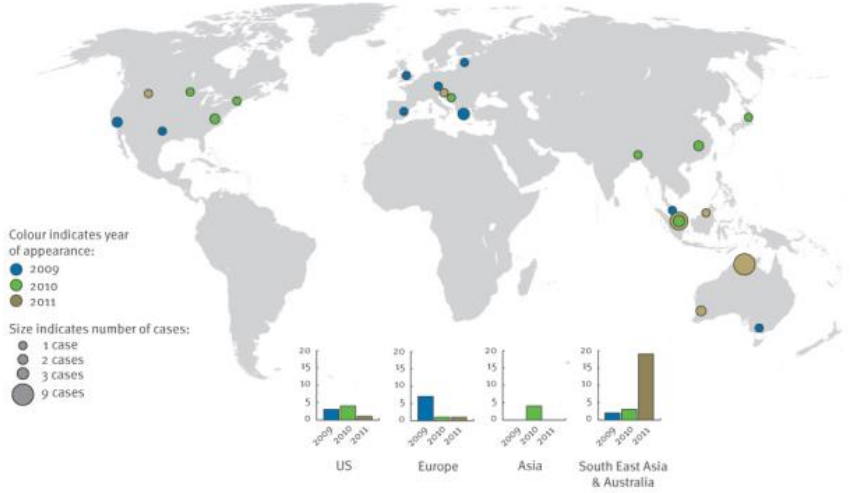


Important disclaimer:

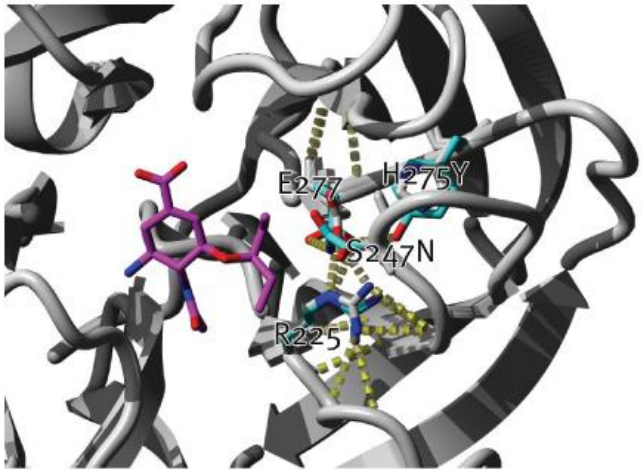
FluSurver makes it very easy to link mutations with prior literature and potential phenotypic effects.

While we have placed great emphasis on avoiding false positive alerts and provide tutorials, one still needs to read the associated papers and interpret the provided evidence carefully to judge any effect realistically.

New drug sensitivity altering mutation NA S247N

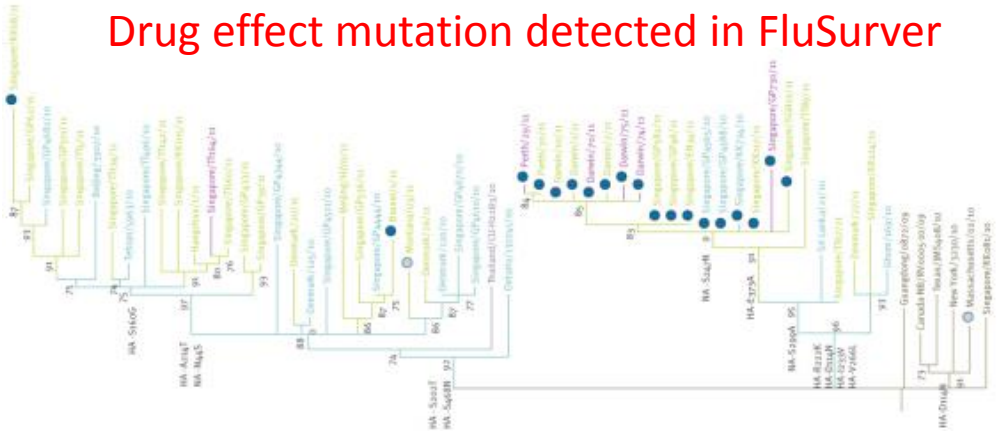


Global occurrence of new variant



Structural context of mutation

Drug effect mutation detected in FluSurver



Phylogenetic context of new variant

Found circulating in 10% of samples in Singapore and 30% of samples in Northern Australia in early 2011.

Experimentally measured increase of IC50 for Tamiflu by 6-fold and Relenza by 3-fold but normally administered dose of drugs still sufficient.

Hurt AC, Lee RT, Leang SK, Cui L, Deng YM, Phuah SP, Caldwell N, Freeman K, Komadina N, Smith D, Speers D, Kelso A, Lin RT, Maurer-Stroh S, Barr IG. *Increased detection in Australia and Singapore of a novel influenza A(H1N1)2009 variant with reduced oseltamivir and zanamivir sensitivity due to a S247N neuraminidase mutation.* Euro Surveill. 2011 Jun 9;16(23). pii: 19884.

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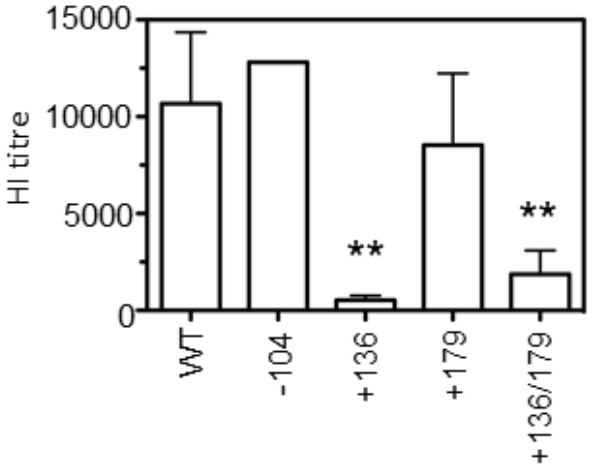
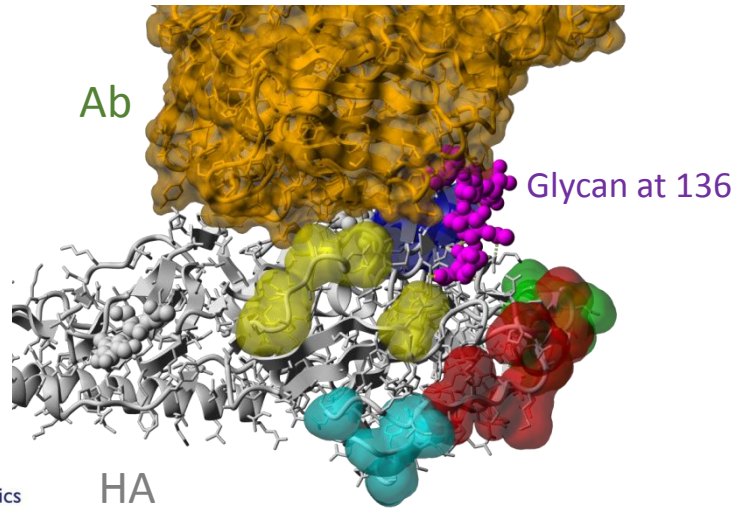
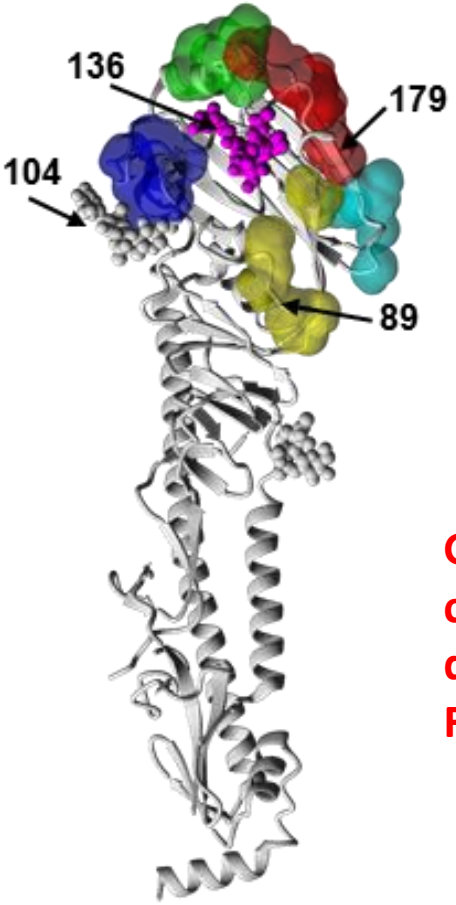
Collaboration with NPHL/Ministry of Health Singapore and WHO Collaborating Centre for Reference and Research on Influenza.

Addition of Glycosylation to Influenza A Virus Hemagglutinin Modulates Antibody-Mediated Recognition of H1N1 2009 Pandemic Viruses. Job ER, Deng YM, Barfod KK, Tate MD, Caldwell N, Reddiex S, Maurer-Stroh S, Brooks AG, Reading PC.
J Immunol. 2013 Mar 1;190(5):2169-77.







Glycosylation change detection in FluSurver

Addition of a glycan to A(H1N1)pdm HA through a K136N mutation was associated with resistance to neutralizing Abs and showed enhanced growth in A(H1N1)pdm-vaccinated mice, consistent with evasion of Ab-mediated immunity in vivo.

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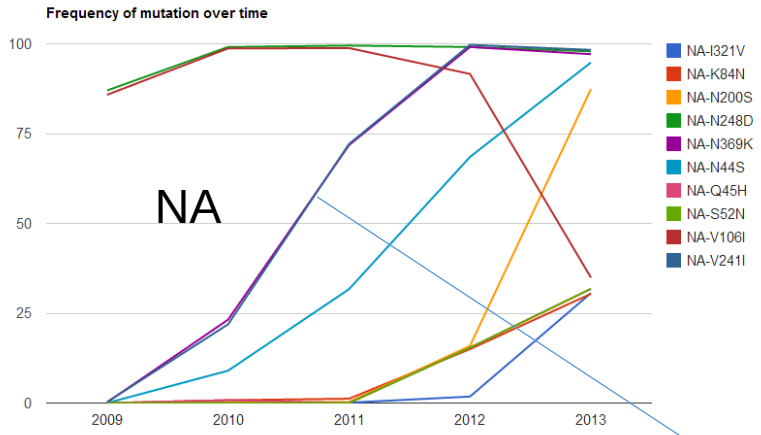
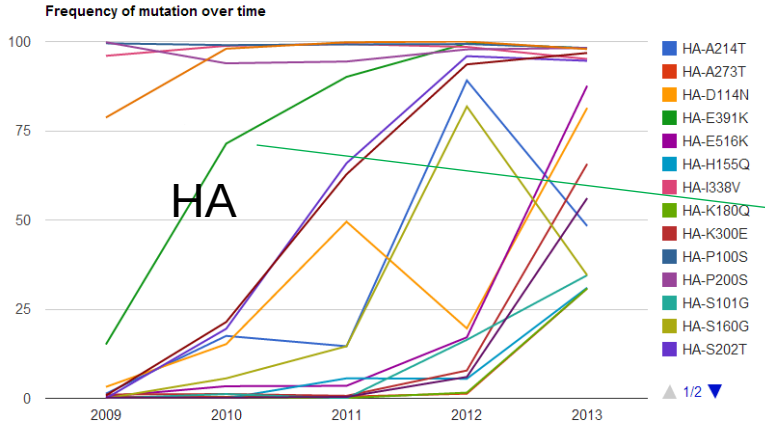
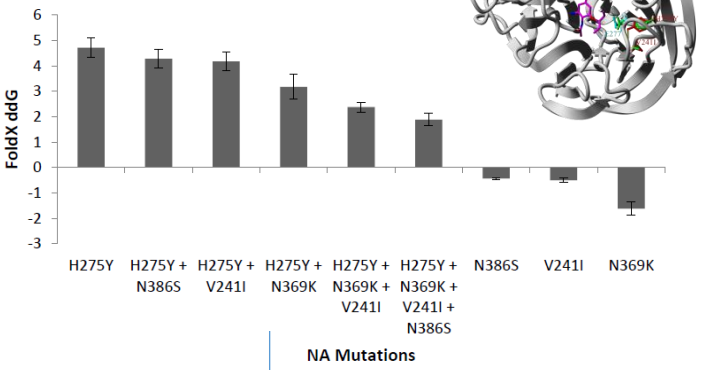
Mutation frequency pattern highlights relevant changes





Temporal frequency plot in FluSurver

FoldX stability for N1pdm in FluSurver

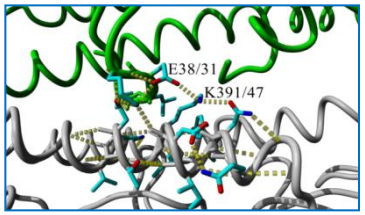


New H275Y permissive mutations

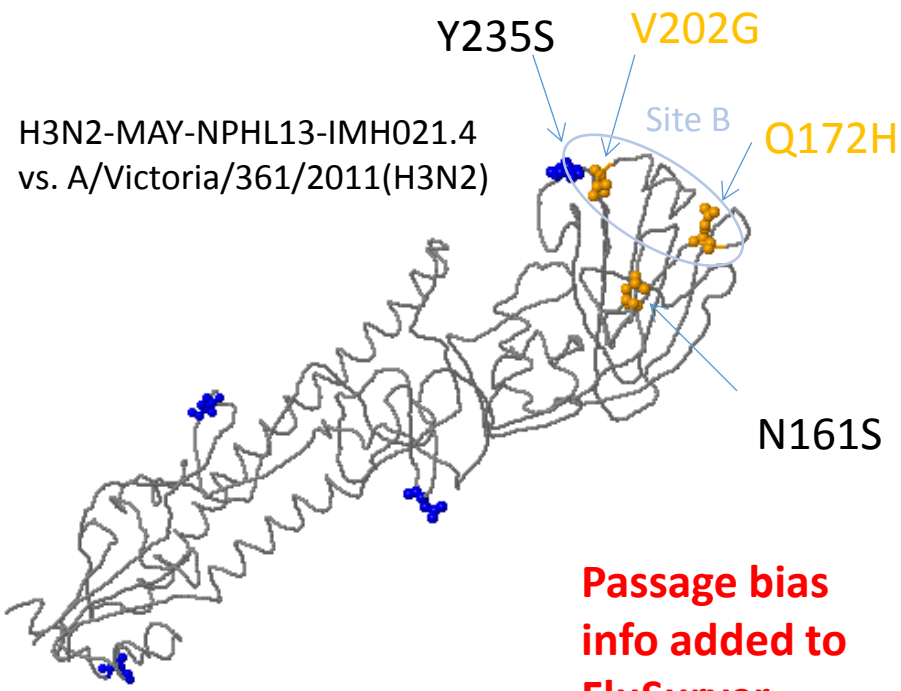
Hurt *et al.* J Infect Dis. 2012 Jul 15;206(2):148-57.
Butler *et al.* PLoS Pathog. 2014 Apr 3;10(4):e1004065.

Change in pH-dependency of fusion

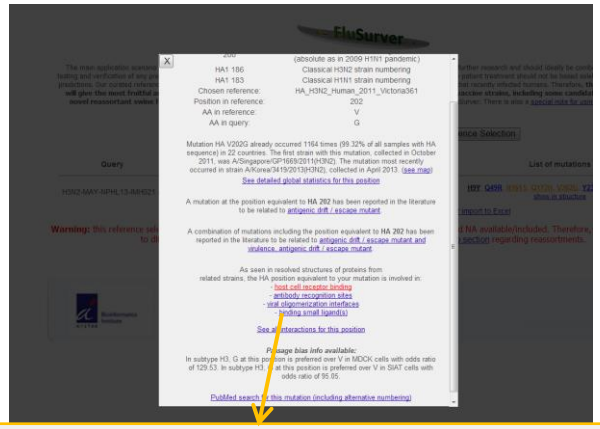
Maurer-Stroh *et al.* PLoS Curr. 2010 Jun 1;2:RRN1162.
Cotter *et al.* PLoS Pathog. 2014 Jan;10(1):e1003831.



Current H3N2 strains with HA passage bias mutations in antigenic sites



Passage bias info added to FluSurver



As seen in resolved structures of proteins from related strains, the HA position equivalent to your mutation is involved in:
- host cell receptor binding
- antibody recognition sites
- viral oligomerization interfaces
- binding small ligand(s)

V202G

See all interactions for this position

Passage bias info available:
In subtype H3, G at this position is preferred over V in MDCK cells with odds ratio of 129.53. In subtype H3, G at this position is preferred over V in SIAT cells with odds ratio of 95.05.

As seen in resolved structures of proteins from related strains, the HA position equivalent to your mutation is involved in:
- host cell receptor binding
- antibody recognition sites
- binding small ligand(s)
- is involved in binding host protein(s)
- viral oligomerization interfaces

Q172H

See all interactions for this position

Passage bias info available:
In subtype H3, H at this position is preferred over Q in SIAT cells with odds ratio of 67.59.

Same isolate but different passage
(A/SINGAPORE/22/2012 NPHL: GP1187-2012)

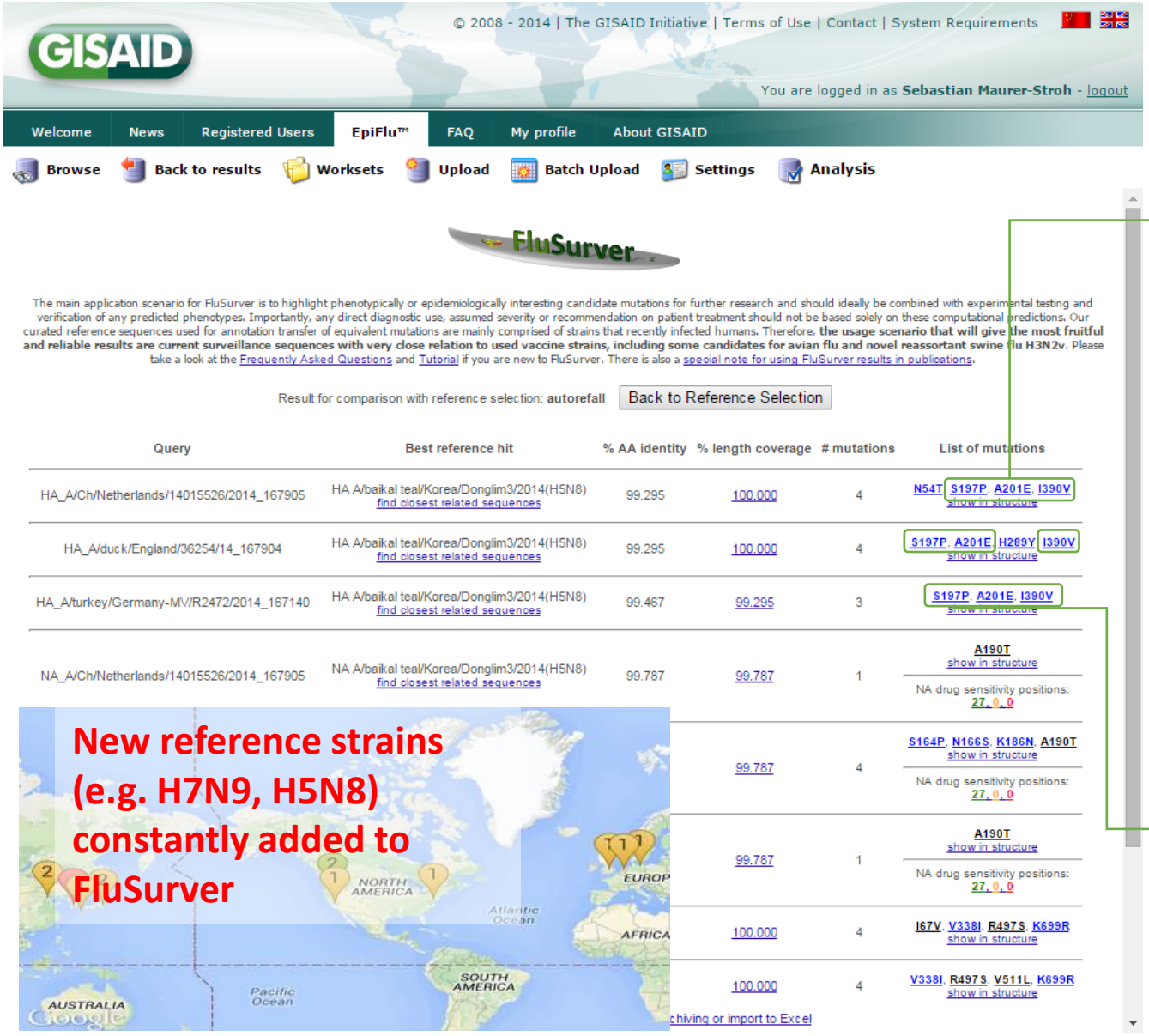
GISAID ID	Submitter	Passage	Mutations relative to Victoria/361
EPI_ISL_128750	WHO CC Melbourne via NPHL	**MDCK0, MDCK1**	H9Y, Q49R, N161S, **Q172H, V202G, Y235S**, N294K
EPI_ISL_135838	US CDC via WHO CC Melbourne	**E4/E1**	H9Y, Q49R, N161S, N294K

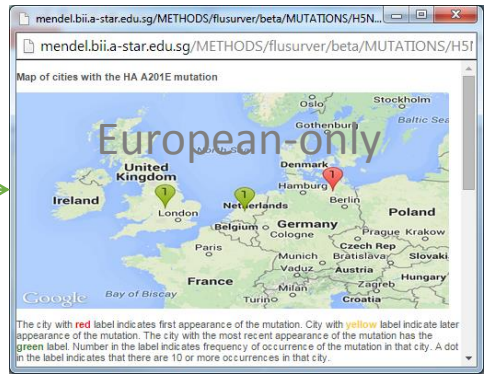
H5N8 sequence analysis with FluSurver@GISAID

Allows detailed analysis of mutations relative to A/baikal teal/Korea/Donglim3/2014(H5N8)

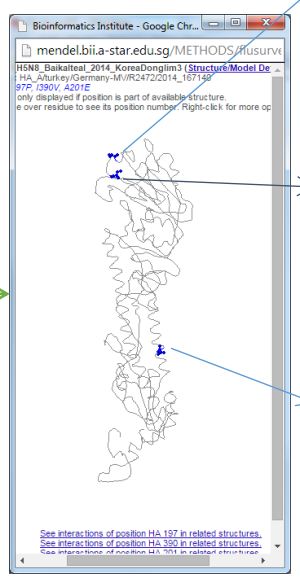
Together with FLI Germany

Emerg Infect Dis. 2015 May;21(5):860-3.





E.g. 3 HA mutations relative to Korean H5N8 shared among European cases



A201E (A189E)
Strong surface change at common epitope (antibody binding site)

S197P (S185P)
Structural change near receptor binding site but effect not yet reported in literature

I390V (I45V)
Conservative change at site recognized by "universal" stem antibodies

H5N8 analysis available on GISAID platform

Summary of FluSurver features

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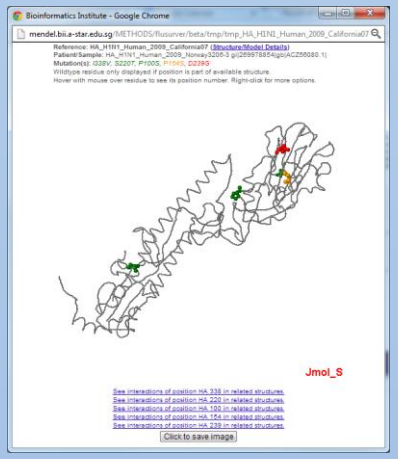
GISAID

Structure

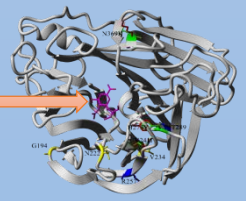


Map mutations to structure

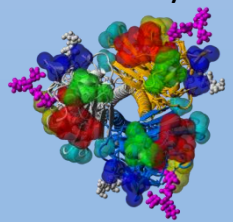
300+ reference homology models

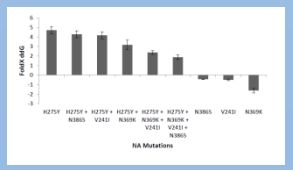
1568	self/oligomerization
975	other small ligand
268	antibody
188	host protein
182	antigen-presenting MHC molecule
132	other viral protein
46	drug
45	nucleic acids
13	host cell receptor
3417	**total interactions for 2062 positions**

Interactions



Glycosylation site changes





FoldX stability calculations

(for high frequency mutations in N1pdm)

Literature

Mutation **numbering scheme conversion** (e.g. H3, H1, H1pdm) and direct **PubMed** search link

Passage bias (egg/cell adaptation) for ~1300 mutations

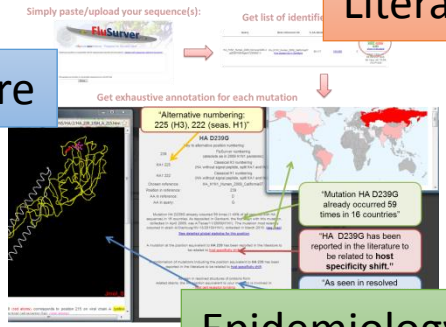
Updated

Literature-curated mutation effect database

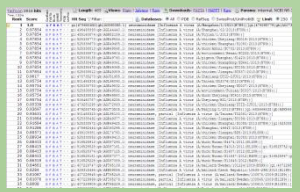
~400 entries

mild drug resistance	30
strong drug resistance	40
virulence	106
antigenic drift / escape mutant	84
host specificity shift	136
other	23

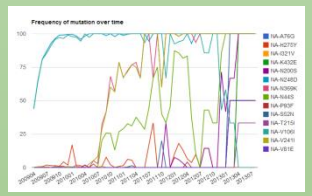
Epidemiology

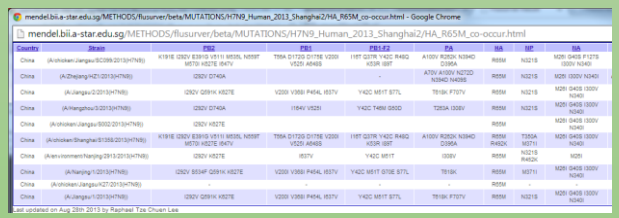


Closest DB hits

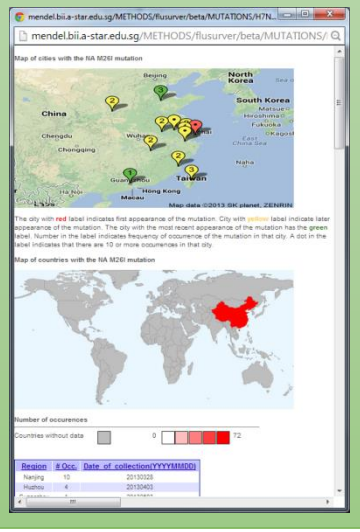


Temporal pattern





Genomic co-occurrence



Regional & global occurrence

Flu work acknowledgements

Many current and former colleagues from the A*STAR Bioinformatics Institute (BII) contribute(d) critically to the FluSurver development and research, including:

Sebastian Maurer-Stroh, **Raphael Tze Chuen Lee, Vithiagaran Gunalan, Vachiranee Limviphuvadh, Fernanda L Sirota, Biruhalem Taye, Alvin Han, Han Hao, Dimitar Kenanov, Jianmin Ma, Swe Swe Thet Paing, Narumol Doungpan, Joy Xiang and Frank Eisenhaber.**

The FluSurver would be nothing without the valuable feedback and interaction with the influenza research and surveillance community, including especially and in chronological order:

- Genome Institute of Singapore (GIS), Singapore
- INMEGEN Mexico City, Mexico
- Experimental Therapeutics Centre (ETC), Singapore
- Tan Tock Seng Hospital (TTSH), Singapore
- National Public Health Laboratory (NPHL) of the Ministry of Health, Singapore
- IAL Sao Paulo, Brazil
- WHO Collaborating Centre for Reference and Research on Influenza, Australia
- Duke-NUS Emerging Infectious Disease Programme, Singapore
- University of Melbourne, Australia
- Global Initiative for Sharing All Influenza Data
- Centers for Disease Control (CDC) Atlanta, USA
- Research and Policy for Infectious Disease Dynamics (RAPIDD)
- Health Protection Agency of Canada
- Friedrich Loeffler Institute, Germany

... and thank all of you!

Fishing for Flu Mutations since 2009!