Vol.54, n. 1: pp.15-23, January-February 2011
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Homology Modelling and Insilico Analysis of Neuraminidase Protein in H1N1 Influenza A Virus

Abhilash Manohar*
Department of Biotechnology Engineering, Oxford College of Engineering, Bangalore - India

ABSTRACT

In this work, modelling of Neuraminidase protein of Influenza A virus (A/Himeji/1/2009(H1N1)) neuraminidase (NA) protein was done using Modeller 9V2. Modelled structure was submitted to protein model database and could be downloaded using accession number PM0075830. The modelled protein structure was subjected to In silco analysis using various bioinformatics tools. Two anti-influenza drugs currently being used to treat infected patients are oseltamivir (Tamiflu) and zanamivir (Relenza), both of which target the neuraminidase enzyme of the virus. Reports of the emergence of drug resistance make the development of new anti-influenza molecules a priority. Hence the modelled structure of H1NI Neuraminidase could be very useful for in silico analysis of potential neuraminidase inhibitors.

Key words: H1N1, Neuraminidase, Modelling, in silico analysis

INTRODUCTION

The 2009 flu pandemic has been a global outbreak of a new strain of influenza A virus subtype H1N1, identified in April 2009 and commonly referred to as swine flu, which infects and is transmitted between humans. It is thought to be a mutation - more specifically, a reassortment - of four known strains of influenza A virus subtype H1N1: one endemic in humans, one endemic in birds, and two endemic in pigs (swine). Swine influenza (also called swine flu, hog flu, and pig flu) is an infection of a host animal by any one of several specific types of microscopic organisms called "swine influenza virus". A June 10, 2009 update by the United Nation's World Health Organization (WHO) states that "74 countries have officially reported 27,737 cases of influenza A (H1N1) infection, including 141 deaths".WHO officially declared the outbreak to be a "pandemic" on June 11, but stressed that the new designation was a result of the global "spread of the virus," not its severity. The WHO stated the pandemic appeared to have moderate severity in comparatively well-off countries however, it would be prudent to anticipate a bleaker picture if the virus spread to areas with limited resources, poor health care, and a high prevalence of underlying medical problems. The case fatality rate (CFR) of the pandemic strain was estimated at 0.4% (range 0.3%-1.5%).

A swine influenza virus (SIV) is any strain of the influenza family of viruses that is usually hosted by (is endemic in) pigs. As of 2009, the known SIV strains was the influenza C virus and the subtypes of the influenza A virus known as H1N1, H1N2, H3N1, H3N2, and H2N3. Swine influenza is common in pigs in the United States (particularly in the midwest and occasionally in other states), Mexico, Canada, South America,

*Author for correspondence: abhibiotek@gmail.com

Europe (including the United Kingdom, Sweden, and Italy), Kenya, and eastern Asia (namely China, Taiwan, and Japan). The 2009 swine flu outbreak in humans was due to a new strain of influenza A virus subtype H1N1 that contained genes closely related to swine influenza (Trifonov et al., 2009). The origin of this new strain is unknown. However, the World Organization for Animal Health (OIE) reported that this strain had not been isolated in pigs (Maria Zampaglione, 2009). This strain can be transmitted from human to human, and causes the normal symptoms of influenza (Myers et al., 2007). Pigs can become infected with human influenza, and this appears to have happened during the 1918 flu pandemic and the 2009 swine flu outbreak.

Virus characteristics

The virus is a novel strain of influenza from which human populations have been neither vaccinated nor naturally immunized. The Centers for Disease Control and Prevention (or CDC), after examining the virus samples from suspected cases in Mexico, matched the strain with those from cases in Texas and California, and found no known linkages to either to animals or one another. It was also determined that the strain contained genes from four different flu viruses: North American swine influenza; North American avian influenza; human influenza; and two swine influenza viruses typically found in Asia and Europe. Further analysis showed that several proteins of the virus were most similar to strains that cause dmild symptoms in humans. Scientists in Winnipeg completed the first full genetic sequencing of the virus on 6 May 2009.

Influenza A

Swine influenza is known to be caused by the influenza A subtypes H1N1 (Shin et al., 2006), H1N2 (Shin et al., 2006), H3N1 (Shin et al., 2006), H3N2 (Ma et al., 2007), and H2N 3(Ma et al., 2007). In pigs, three influenza A virus subtypes (H1N1, H3N2, and H1N2) are the most common strains worldwide (Ma et al., 2007). In the United States, the H1N1 subtype was exclusively prevalent among the swine populations before 1998; however, since late August 1998, H3N2 subtypes have been isolated from the pigs. As of 2004, H3N2 virus isolates from US swine and turkey stocks were triple reassortants, containing genes from human (HA, NA, and PB1), swine (NS, NP, and M), and avian (PB2 and PA) lineages (Gramer et al., 2007).

Virus origins

In early June, Oxford University's Department of Zoology, reported test results that showed that this strain has been circulating among pigs, possibly among multiple continents, for many years prior to its transmission to humans. The research team that worked on this report also reported that it was derived from several viruses circulating in swine, and that the initial transmission to humans occurred several months before recognition of the outbreak. The team concluded that despite widespread influenza surveillance in humans, the lack of systematic swine surveillance allowed for the undetected persistence and evolution of this potentially pandemic strain for many years (Smith et al., 2009). According to the findings, the movement of live pigs between Eurasia and North America seemed to have facilitated the mixing of diverse swine influenza viruses, leading to the multiple reassortment events associated with the genesis of the (new H1N1) strain (Lindstrom et al., 2004).

Transmission of swine influenza virus from pigs to humans is not common and does not always cause human influenza, often only resulting in the production of antibodies in the blood. The meat of the animal poses no risk of transmitting the virus when properly cooked. If transmission does cause human influenza, it is called zoonotic swine flu. People who work with pigs, especially people with intense exposures, are at increased risk of catching swine flu. In the mid-20th century, the identification of influenza subtypes became possible, which allows accurate diagnosis of transmission to humans. Since then, fifty confirmed transmissions have been recorded.

Rarely, these strains of swine flu can pass from human to human. In humans, the symptoms of swine flu are similar to those of influenza and of influenza-like illness in general, namely chills, fever, sore throat, muscle pains, severe headache, coughing, weakness and general discomfort (Kimura et al., 2008).

Treatment

Antiviral drugs

According to the CDC, the antiviral drugs can be given to treat those who become severely ill. These antiviral drugs are prescription medicines

(pills, liquid or an inhaler) and act against influenza viruses, including the 2009 pandemic virus. There are two such medications that are recommended for use against the 2009 H1N1 swine flu virus, oseltamivir (Tamiflu) and zanamivir (Relenza) (Antonovics et al., 2006). CDC has noted that as the flu pandemic spreads, antiviral drugs such as oseltamivir (Tamiflu) and zanamivir (Relenza) might become in short supply. Therefore, the drugs would be given first to those people who have been hospitalized or are at high risk of complications(Olsen, 2002).The drugs work best if given to the patient within two days of becoming ill , but might be given later if illness became severe or to those at a high risk for complications.

Neuraminidase

Neuraminidase is an enzyme on the surface of influenza viruses that enables the virus to be released from the host cell. When influenza virus reproduces, it moves to the cell surface with a hemagglutinin molecule on the surface of the virus bound to a sialic acid receptor on the surface of the cell. In order for the virus to be released free from the cell, neuraminidase must break apart (cleave) the sialic acid receptor.

Function

The enzyme helps viruses to be released from a host cell. Influenza virus membranes contain two glycoproteins: haemagglutinin and neuraminidase. While the hemagglutinin on the surface of the virion is needed for infection, its presence inhibits the release of the particle after budding. It also mediates cell-surface sialic acid receptor binding to initiate virus infection. Viral neuraminidase cleaves the terminal neuraminic acid (also called sialic acid) residues from glycan structures on the surface of the infected cell. This promotes the release of progeny viruses and the spread of the virus from the host cell to uninfected surrounding cells. Neuraminidase also cleaves sialic acid residues from viral proteins, preventing the aggregation of viruses.

MATERIAL AND METHODS

Influenza A virus (A/Himeji/1/2009(H1N1)) segment 6 neuraminidase (NA) sequence with accession number GQ261273 Submitted (15-JUN-2009) by Horikawa et al., from National Institute of Technology and Evaluation (NITE), Tokyo, Japan was selected for in silico analysis.

The Sequence selected for the analysis

```
>MNPNQKIITIGSVCMTIGMANLILQIGNIISIWISHSIQLGNQNQIETCNQSVITYENNTWVNQTYVNISNTNFAAGQ
SVVSVKLAGNSSLCPVSGWAIYSKDNSIRIGSKGDVFVIREPFISCSPLECRTFFLTQGALLNDKHSNGTIKDRSPYRT
LMSCPIGEVPSPYNSRFESVAWSASACHDGINWLTIGISGPDNGAVAVLKYNGIITDTIKSWRNNILRTQESECACVNG
SCFTVMTDGPSNGQASYKIFRIEKGKIVKSVEMNAPNYHYEECSCYPDSSEITCVCRDNWHGSNRPWVSFNQNLEYQIG
YICSGIFGDNPRPNDKTGSCGPVSSNGANGVKGFSFKYGNGVWIGRTKSISSRNGFEMIWDPNGWTGTDNNFSIKQDIV
GINEWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKENTIWTSGSSISFCGVNSDTVGWSWPDGAELPFTIDK
```

Tool used for modelling of neuraminidase protein was Modeller 9V2.

Tools used for neuraminidase protein analysis

- Gor IV secondarystructure prediction tool (Garnier et al., 1978): The GOR (Garnier-Osguthore- Robson) method uses both information theory and Bayesian statistics for predicting the secondary structure of proteins.
- Pep tool (Version 2.0): Pep Tool is a suite of applications for comprehensive analysis of peptide and protein sequences.

RESULTS AND DISCUSSION

The MODELLER was used for homology or comparative modeling of three-dimensional protein structures. The Alignment of a sequence to be modelled was provided with the known related structures and the MODELLER automatically calculated a model containing all non-hydrogen atoms. The MODELLER implemented the comparative protein structure modeling by satisfaction of spatial restraints, and could perform many additional tasks, including de-novo modeling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc.

The Structure of modeled protein was visualized using Rasmol (Structure visualization tool)

(Figure 1). Modelled structure was submitted to protein model database (PMDB) a repository for three dimensional protein models obtained by structure prediction methods. The Submitted, Modelled H1N1 Neuraminidase protein could be downloaded from PMDB using accession number PM0075830**.**

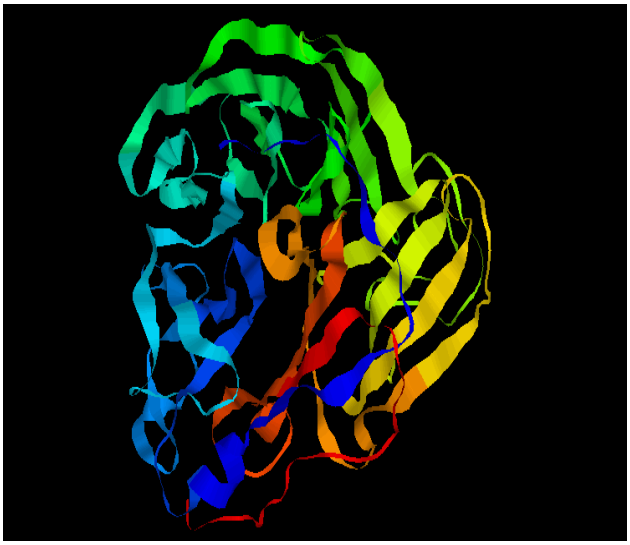

Figure 1 - Structure of modelled neuraminidase protein of H1N1 Influenza virus.

```
10        20        30        40        50        60        70
          |         |         |         |         |         |         |
MNPNQKIITIGSVCMTIGMANLILQIGNIISIWISHSIQLGNQNQIETCNQSVITYENNTWVNQTYVNIS
ccccccceeeeceeeeeeccchhhhhcceeeeeeeccccccccccceeccceeeeeccccceeeeeeccc
NTNFAAGQSVVSVKLAGNSSLCPVSGWAIYSKDNSIRIGSKGDVFVIREPFISCSPLECRTFFLTQGALL
ccccccccceeeeeeccccccccccceeeeccccceeeccccceeeeeccceeccccccccccceeccchhh
NDKHSNGTIKDRSPYRTLMSCPIGEVPSPYNSRFESVAWSASACHDGINWLTIGISGPDNGAVAVLKYNG
hccccccccccccceeeeeccccccccccccceeeeeceeeeccccceeeeeeccccccceeeeeeeccc
IITDTIKSWRNNILRTQESECACVNGSCFTVMTDGPSNGQASYKIFRIEKGKIVKSVEMNAPNYHYEECS
eeeeeeeeeeeeeeeccceeeeeccceeeeeeccccccchhhhhhhhhhccceeeeeeeccccceeceeeee
CYPDSSEITCVCRDNWHGSNRPWVSFNQNLEYQIGYICSGIFGDNPRPNDKTGSCGPVSSNGANGVKGFS
ccccccceeeeeccccccccccceeeeccceeeeeeeeeeeeccccccccccccccccceeccccccceeee
FKYGNGVWIGRTKSISSRNGFEMIWDPNGWTGTDNNFSIKQDIVGINEWSGYSGSFVQHPELTGLDCIRP
eeccceeeeeccceecccceeeeeeccccccccccccccceeeeeeeccccccceeeccccccccccc
CFWVELIRGRPKENTIWTSGSSISFCGVNSDTVGWSWPDGAELPFTIDK
cccceeeccccccceeeeeeccceeeeeeeeccceeccccccccceeeeeec
```

```
Sequence length :   469
GOR4 :

   Alpha helix     (Hh) :    18 is   3.84%
   3_10 helix      (Gg) :     0 is   0.00%
   Pi helix        (Ii) :     0 is   0.00%
   Beta bridge     (Bb) :     0 is   0.00%
   Extended strand (Ee) :   197 is  42.00%
   Beta turn       (Tt) :     0 is   0.00%
   Bend region     (Ss) :     0 is   0.00%
   Random coil     (Cc) :   254 is  54.16%
   Ambigous states (?)  :     0 is   0.00%
   Other states         :     0 is   0.00%
```

Protein Structure Analysis

The Secondary structure prediction of the modelled neuraminidase virulence protein was carried out using GOR IV (Garnier-Osguthore-Robson) secondary structure prediction tool (Figure 2).

Amino acid frequency plot (Figure 3), plot of charge vs pH (Figure 4), Beta staircase model (Figure 5), Helical wheel model (Figure 6) and molecular properties calculation (Table 1) of the neuraminidase protein of H1N1 Influenza virus was obtained using pep tool a comprehensive protein analysis software.

Beta staircase Model

The beta staircase graphically displays (Figure 5) the disposition of amino acid side chains about an assumed alpha helix. The view is always along the central axis of the helix from N to C-terminus. The helical wheel is an effective method for displaying the symmetry of hydrophobic/hydrophilic side chains of BBI C-II. It is useful for observing how the amino acids are positional in relation to one another (Khot et al., 2004)

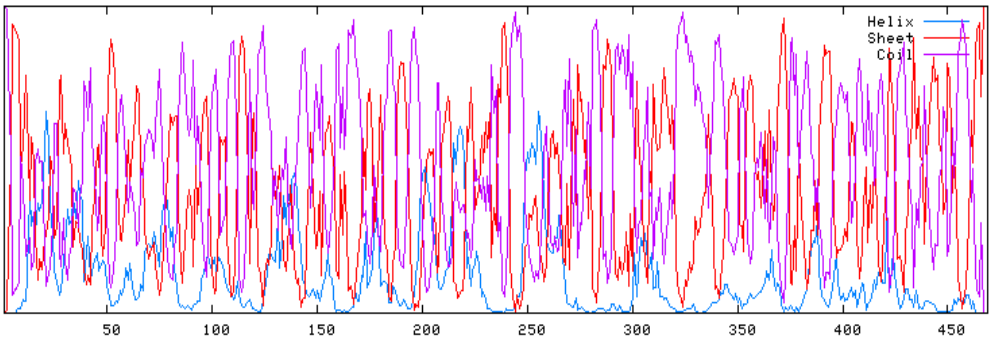

Figure 2 - GOR IV (Garnier- Osguthore- Robson) secondary structure prediction of Neuraminidase protein of H1N1 Influenza virus.

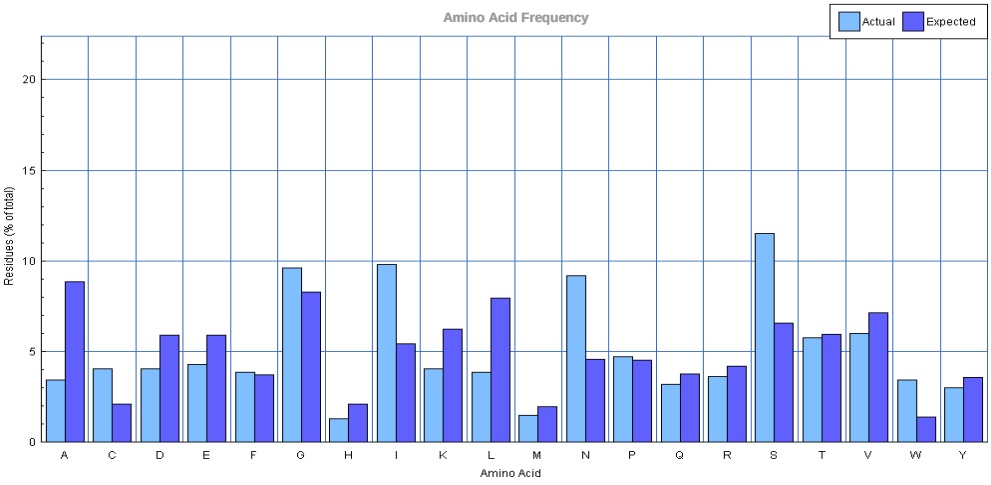

Figure 3 - Amino acid frequency plot of amino acids (Actual and Expected) of neuraminidase protein of H1N1 Influenza virus.

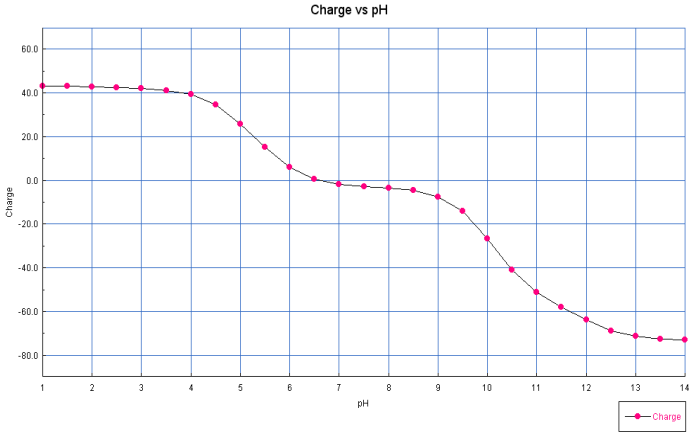

Figure 4 - Plot of charge vs pH

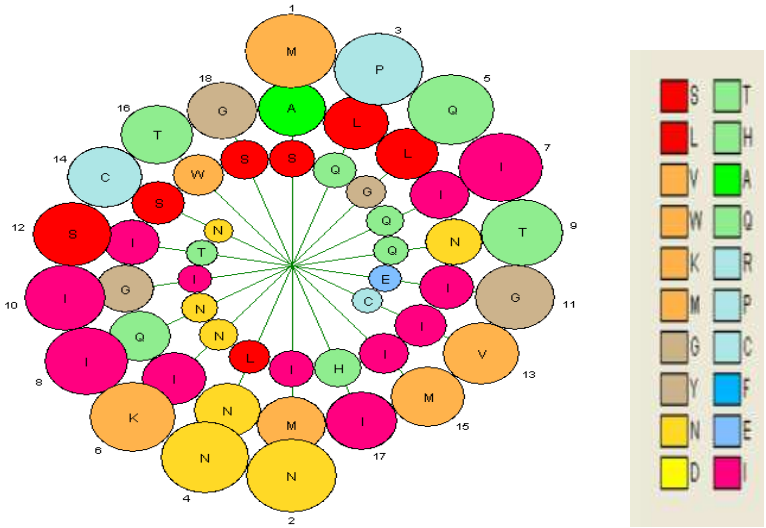

Figure 5 - Beta staircase model of neuraminidase protein of H1N1 influenza virus.

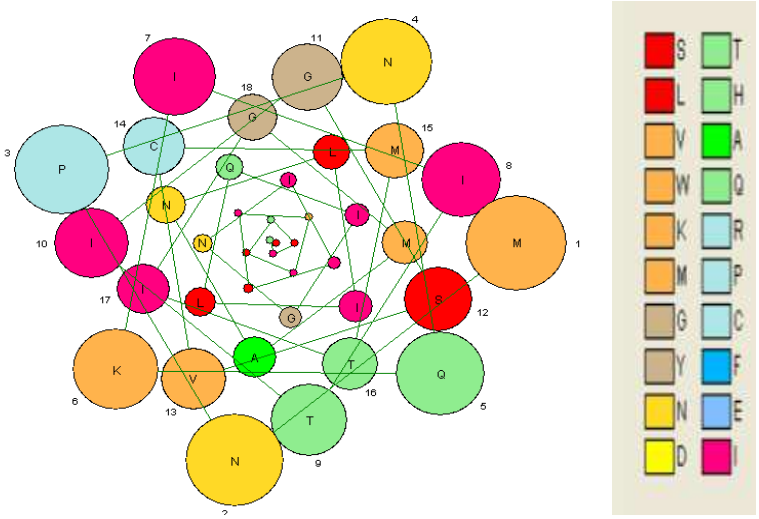

Figure 6 - Helical wheel model of neuraminidase protein of H1N1 influenza virus.

Table 1 - Molecular properties calculation of neuraminidase protein of H1N1 influenza virus.

Protein Property	Score	Description of property
Molecular weight (Daltons)	51611.133	The sum total atomic weight for all the amino acids comprising the current sequence. Molecular weight calculations do not take into account post translational modifications such as N- and C-terminal modifications or glycosylated residues.
Number of Amino acids	469	The total number of amino acids comprising the current sequence.
Mean amino acid weight (Daltons)	110.045	The average molecular weight of the amino acids comprising the current sequence. The mean amino acid weight is calculated simply as the molecular weight divided by the number of amino acids in the sequence.
Average hydrophobicity	-0.264819	The average hydrophobicity (AH) is the sum of all hdrophobicity values for the given sequence divided by its sequence length. The hydrophobicity values from Kyte/Doolittle are used in this calculation.
Ratio of hydrophilicity to hydrophobicity	1.23302	The ratio of hydrophobic to hydrophobic amino acids. RH=1.22 indicates an average protein, RH>1.90 indicates a non-folding protein,RH<0.85 indicates an insoluble protein.
Percentage of Hydrophilic Amino acids	51.5991	The percentage of hydrophilic amino acids comprising the current sequence. For naturally occurring soluble proteins, the average percentage of hydrophilic amino acids is: 47.56%. The hydrophilic amino acids (Kyte- Doolittle hydropathy values) are: DEHKNPQRST.
Percentage of Hydrophobic Amino acids	48.4008	The percentage of hydrophilic amino acids comprising the current sequence. For naturally occurring soluble proteins, the average percentage of hydrophobic amino acids is 52.44 %. The hydrophobic amino acids (Kyte- Doolittle hydropathy values) are: ACFGILMVWY.
Ratio of % hydrophilic to hydrophobic	1.06608	This is an indicator of the protein sequence's propensity to fold into a globular structure in normal physiological conditions. RHP=0.91 indicates average protein. RHP>1.42 indicates a non-folding protein. RHP<0.77 indicates an insoluble protein.
Mean Beta hydrophobic moment	0.185993	The mean beta hydrophobic moment is the sum of all beta hydrophobic moment values for the given sequence divided by its sequence length. The hydrophobic moment values from Cornette are used in this calculation.
Mean helix Hydrophobic Moment	0.15933	The mean helix hydrophobic moment is the sum of all helix hydrophobic moment values for the given sequence divided by its sequence length. The hydrophobic moment values from Cornette are used in this calculation.
Number of Basic Amino acids	36	The sum total of Arginine(R) and Lysine (K) residues comprising the current sequence. Basic amino acids carry a net positive charge at physiological Ph (7.2)
Number of acidic Amino acids	39	The sum total of aspartic acid (D) and glutamic acid (E) residues comprising the current sequence. Acidic amino acids carry a net negative charge at physiological Ph (7.2).
Total linear charge density	0.0912951	The total number of charged amino acids (K,R,D, and E), plus the N- and C-terminal groups, divided by the total number of amino acids in the protein sequence. The total linear charge density is a measure of the potential solubility of a protein; values greater than about 0.2 are typically required for a protein to be soluble.
Estimated Pi for protein	6.6	The pH at which the protein carries a net zero charge. Peptides and proteins at their isoelectric point tend to be somewhat insoluble.
Polar area of extended chain (Angs^2)	30435.4	The summation of polar surface area (ASAp) for each of the amino acids comprising the current sequence, assuming an extended structure (in square angstroms). ASAp values are attributed to unchanged nitrogen, oxygen and sulphur atoms, which are considered to be polar.
Non Polar area of extended chain (Angs^2)	50124.8	The summation of the non-polar surface area (ASAp) for each of the amino acids comprising the current sequence, assuming an extended structure (in square angstroms). ASAnp values are attributed to carbon atoms, which are considered to be non-polar.
Total area of extended chain (Angs^2)	80580.2	The summation of charged, polar and non-polar accessible surface area for each of the amino acids comprising the current sequence (In square Angstroms).
Polar accessible surface area of Folded Protein (Angs^2)	6993.02	The polar accessible surface area for the amino acids comprising the current sequence, assuming the protein folds into globular structure (in square Angstroms).

(cont. ...)

(cont. Table 1)

Protein Property	Score	Description of property
Non Polar accessible surface area of Folded Protein (Angs^2)	10206.3	The non-polar accessible surface area for the amino acids comprising the current sequence, assuming the protein folds into globular structure (in square Angstroms).
Total Accessible surface area of Folded Protein (Angs^2)	17199.3	The total accessible surface area for the amino acids comprising the current sequence, assuming the protein folds into a globular structure.
Ratio of folded to extended area	0.225184	The value for the estimated accessible surface area of an assumed globular folded protein to that of the extended chain.
Buried polar area of folded protein (Angs^2)	20658.8	The total polar area of the folded protein that is not accessible to solvent, assuming a globular protein structure (in square Angstroms). The ABP is assumed to be 35% of the total buried surface area.
Buried non polar area of folded protein (Angs^2)	36001.9	The total Non-polar area of the folded protein that is not accessible to solvent, assuming a globular protein structure (in square Angstroms). The ABN is assumed to be 61% of the total buried surface area.
Buried Charge Area of FP	2360.78	The total charged area of the folded protein that is not accessible to solvent, assuming a globular protein structure (in square Angstroms). The ABC is assumed to be 4% of the total buried surface area.
Total Buried Surface (Angs^2	58127.3	The total charged area of the folded protein that is not accessible to solvent, assuming a globular protein structure (in square Angstroms). The AB is defined as the total area of the extended chain minus the accessible surface area of the folded protein.
Number of Buried Amino Acids	193	The number of amino acids that have less than 5% surface area accessible to solvent, assuming the protein forms a globular structure. Average NB% for small proteins (<100aa): 15% Average NB% for small proteins (>100aa):32%
Packing Volume (est) (Angs^3)	61771.7	This value is a rough estimate of the packing volume (in cubic Angstroms) calculated from the molecular weight of the current sequence. Estimated packing volume (VPe) is defined as 1.245*molecular weight. This value assumes the protein forms a globular, spherical structure.
Interior Volume of Protein (Angs^3)	44594.8	The volume occupied by the fraction of amino acids estimated to be hidden from the solvent (in cubic Angstroms).
Exterior Volume of Protein (Angs^3)	16197	The volume occupied by the fraction of amino acids estimated to be accessible to the solvent (in cubic Angstroms).
Partial Specific Volume (ml/g)	0.715096	The sum of the partial specific volumes multiplied by the weight percent, for each of the individual amino acids comprising the protein sequence. PSVs may be useful in determining a protein's retention time during size- exclusion chromatography, or in ultra-centrifugation studies.
Fisher Volume Ration (act)	0.363203	If FVR (act) >FVR (idealized) the molecule likely forms soluble monomer. If FVR (act) >> FVR Ratio (idealized) the molecule likely doesn't fold into compact structure. If FVR (act) < FVR (idealized) the molecule likely aggregates.
Fisher Volume Ratio (idealized)	0.533676	If FVR (act) >FVR (idealized) the molecule likely forms soluble monomer. If FVR (act) >> FVR Ratio (idealized) the molecule likely doesn't fold into compact structure. If FVR (act) < FVR (idealized) the molecule likely aggregates.
Protein Solubility	1.42657	A relative measure of a protein's solubility based on hydrophobicity and acharge data. Solublity=1.6 indicates an average protein. Solubilty < 1.1 indicates an insoluble protein.
Est. Radius of Folded Protein (Angs)	30.1067	The estimated radius, in Angstroms, for the current sequence, assuming it folds into a globular protein. The radius is defined as the cube root of the number of amino acids comprising the sequence multiplied by the average distance between adjacent amino acid c-alpha atoms (3.875 Angstroms).
RSM End to End Dist. Of Ext. Chain(Angs)	227.134	The root-mean-square (RMS) distance, from N-C-terminus, for the protein sequence assuming an extended structure. The RMS distance is in Angstroms.
Radius of Gyration of Ext. Chain (Angs)	92.7272	The root-mean-square (RMS) radius of the unfolded, extended protein chain from its center of gravity.
Solvent Free Energy of Folding (kcal/mol)	-448.29	The estimated Gibbs free energy difference (in Kcal/mol) between the extended, unfolded chain and an assumed globular, folded protein. Negative SFEs correspond to a stabilizing solvent effect upon folding of the extended chain into the globular form.

CONCLUSIONS

Novel H1N1 (Referred to as “Swine flu” early on) is a new influenza virus causing illness in people. This new virus was first detected in people in the US in April 2009. A June 10, 2009 update by the WHO state that 74 countries had officially reported 27,737 cases of influenza A (H1N1) infection, including 141 deaths. Two anti-influenza drugs currently being used to treat the infected patients are oseltamivir (Tamiflu) and zanamivir (Relenza), both of which target the neuraminidase enzyme of the virus. Reports of the emergence of drug resistance make the development of new anti-influenza molecules a priority. This project aimed at designing structure of Neuraminidase of H1N1 which will be useful for designing the novel Neuraminidase inhibitors which might help to combat H1N1 pandemic.

REFERENCES

Antonovics J, Hood ME, Baker CH (April 2006). "Molecular virology: was the 1918 flu avian in origin?". *Nature* 440.

Garnier J., Osguthrope, D.J. Robson, (1978),J.Mol. Bio., **130**, 97-123.

Gramer Marie René, Lee Jee Hoon, Choi Young Ki, Goyal Sagar M, Joo Han Soo (July 2007). "Serologic and genetic characterization of North American H3N2 swine influenza A viruses". *Canadian Journal of Veterinary Research* 71 (**3**): 201–206.

Khot S.S., Gomase V.S., Chavan V.M., Hasabe R.P., Ingale A.G., Chikhale N.G.,2004: Structural analysis and comparative modeling of Auxin binding protein rom *Gossypium hirsutum* (Cotton). *Bioinformatics India.*, 2 (**3**): 53-57.

Kimura K, Adlakha A, Simon PM (March 1998). "Fatal case of swine influenza virus in an immunocompetent host". *Mayo Clinic Proceedings. Mayo Clinic* 73 (**3**): 243–5.

Lindstrom Stephen E, Cox Nancy J, Klimov Alexander (15 October 2004). "Genetic analysis of human H2N2 and early H3N2 influenza viruses, 1957–1972: evidence for genetic divergence and multiple reassortment events". *Virology* 328 (**1**): 101–19.

Maria Zampaglione (April 29, 2009). "Press Release: A/H1N1 influenza like human illness in Mexico and the USA: OIE statement". World Organisation for Animal Health. http://www.oie.int/eng/press/en_090427.htm. Retrieved on April 29, 2009.

Ma W, Vincent AL, Gramer MR, Brockwell CB, Lager KM, Janke BH, Gauger PC, Patnayak DP, Webby RJ, Richt JA (26 December 2007). "Identification of H2N3 influenza A viruses from swine in the United States". *Proc Nat Acad Sci USA* 104 (52): 20949–54.

Myers KP, Olsen CW, Gray GC (April 2007). "Cases of swine influenza in humans: a review of the literature". *Clin Infect Dis* 44 (8): 1084–8.

Olsen CW (May 2002). "The emergence of novel swine influenza viruses in North America". *Virus Research* 85 (**2**): 199–210.

Shin JY, Song MS, Lee EH, Lee YM, Kim SY, Kim HK, Choi JK, Kim CJ, Webby RJ, Choi YK (2006). "Isolation and characterization of novel H3N1 swine influenza viruses from pigs with respiratory diseases in Korea". *Journal of Clinical Microbiology* 44 (**11**): 3923–7.

Smith GJD, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, Ma SK, Cheung CL, Raghwani J, Bhatt S, Peiris JSM, Guan Y and Rambaut A (11 June 2009). "Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic". *Nature* 459: 1122

V Trifonov, H Khiabanian, B Greenbaum, R Rabadan (30 April 2009). "The origin of the recent swine influenza A (H1N1) virus infecting humans". *Eurosurveillance* 4 (17).

Received: July 18, 2009;
Revised: September 17, 2009;
Accepted: April 16, 2010.