Title

EXPLORING THE BINDING BETWEEN AVIAN INFLUENZA H5N1 VIRUS 3F5T &TRANSMEMBRANE RECEPTOR PROTEIN(4KDO)

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Abstract

The H5N1 influenza A virus that is currently circulating in Asia, Africa and Europe has resulted in persistent outbreaks in poultry with sporadic transmission to humans. Thus far, it is believed that H5N1 does not possess sufficient ability for human-to-human transmission and subsequent pandemic infection. Both receptor binding specificity and virus infectivity are key factors in determining whether influenza A virus becomes pandemic. The use of human viral isolates in various studies has helped to illustrate the changes in receptor binding specificity and virulence as a result of adaptation in humans. In this review, we highlight the important amino acids and domains of viral proteins related to receptor binding specificity that have been reported for humans and avians using mammalian models. Thus, this review will consolidate findings from studies that have shed light on the receptor binding and transmission characteristics of the H5N1 influenza virus, with the goal of improving our ability to predict the transmission efficiency or pandemic potential of new viral strains.

Keywords: receptor binding, transmission, H5N1, mammal, influenza A virus, A/H5N1 influenza virus,A/H7N9 influenza virus, airborne transmission, pathogenesis, receptor binding specificity
Subject Categories: Microbiology, Virology & Host Pathogen Interaction

INTRODUCTION

One important aspect of influenza virus infection is the interaction between the viral surface glycoprotein HA and the corresponding receptor on host cells. To infect host cells, influenza virus utilizes HA to bind to complex glycans on the host cell surface via a terminal sialic acid (SA). Influenza viruses have different preferences for SAs with different linkages. For example, human influenza virus prefers sialic acid linked to galactose via an α-2,6 bond (SAα-2,6Gal), whereas avian influenza virus prefers the terminus with sialic acid linked to galactose via an α-2,3 bond (Saα-2,3Gal). SAα-2,6Gal is the major linkage for vicinal galactose in the human upper respiratory epithelium. Epithelial cells in the paranasal sinuses, pharynx, trachea and bronchi mainly express SAα-2,6Gal, which is also expressed in ciliated and goblet cells in the human lung.Apart from SAα-2,6Gal, the human respiratory tract also expresses SAα-2,3Gal on non-ciliated cuboidal bronchiolar cells, which are situated at the junction between the respiratory bronchiole and alveolus.Highly pathogenic avian H5N1 virus labeled by fluorescein isothiocyanate (FITC) was shown to preferentially attach to type-II pneumocytes, alveolar macrophages, and non-ciliated cuboidal epithelial cells in the terminal bronchioles of the human lower respiratory tract. The binding of H5N1 virus rarely occurs at the trachea and upper respiratory tract, which is consistent with pathological findings observed at autopsy, such as diffuse alveolar damage, interstitial pneumonia, focal hemorrhage, and bronchiolitis.

Influenza virus zoonoses and pandemics

Influenza A viruses can infect a wide range of hosts, including humans, birds, pigs, horses, and marine mammals (Websteretal,1992). Influenza A viruses are classified based on the antigenic properties of the major surface glycoproteins hemagglutinin (HA) and

neuraminidase (NA). To date, 18 HA and 11 NA subtypes have been described. All subtypes have been found in wild aquatic birds except for the recently discovered H17N10 and H18N11 viruses, which have only been detected in bats (Webster et al,1992; Fouchier et al,2005; Tong et al,(2012.2013) Throughout recent history, avian-origin influenza viruses have crossed the species barrier and infected humans. Some of these zoonotic events resulted in the emergence of influenza viruses that acquired the ability to transmit between humans and initiate a pandemic. Four pandemics were recorded in the last century: the 1918 H1N1 Spanish pandemic, the 1957 H2N2 Asian pandemic, the 1968 H3N2 Hong Kong pandemic, and the 2009 H1N1 pandemic (pH1N1) that was first detected in Mexico (reviewed in Sorrell et al,2011). Various other influenza A viruses of pig and avian origin (e.g., of subtypes H5, H6, H7, H9, and H10) have occasionally infected humans—sometimes associated with severe disease and deaths—but these have not become established in humans.

The H5N1 avian influenza virus that was first detected in Hong Kong in 1997 has frequently been reported to infect humans and cause serious disease. As of October 8, 2013, WHO has been informed of 641 human cases of infection with H5N1 viruses, of which 380 died (www.who.int/influenza/human_animal_interface/en/). The majority of these cases occurred upon direct or indirect contact with infected poultry. Due to the high incidence of zoonotic events, the enzootic circulation of the virus in poultry, and the severity of disease in humans, the H5N1 virus is considered to pose a serious pandemic threat. Fortunately, sustained human-to-human transmission has not been reported yet (Kandunet al,2006; Wang et al, 2008).

A more recent example of a major zoonotic influenza A virus outbreak started in China in early 2013. This outbreak, caused by an avian H7N9 virus, resulted in 137 laboratory-confirmed human cases of infection and 45 deaths (www.who.int/influenza/human_animal_interface/en/). Although one case of possible human-to-human transmission was described, thus far, this outbreak also has not lead to sustained transmission between humans (Gao et al, 2013b; Qi et al, 2013). Some mild cases of infection were reported, but the Chinese H7N9 influenza viruses frequently caused severe illness, characterized by severe pulmonary disease and acute respiratory distress syndrome (Gao et al, 2013a,b). Although influenza viruses of the H7 subtype have sporadically crossed the species barrier in the past, with outbreaks reported, for example, in the United Kingdom, Centers for Disease Control and Prevention (2004), Canada (Tweed et al, 2004), the Netherlands (Fouchier et al, 2004), and Italy (Puzelli et al, 2005; www.who.int/influenza/human_animal_interface/en/), the 2013 H7N9 virus appeared to jump the species barrier more easily and was generally associated with more serious disease in humans. Interestingly, the internal genes of the H7N9 virus belong to the same genetic lineage as the internal genes of the H5N1 virus; both are derived from H9N2 viruses (Guan et al, 1999; Lam et al, 2013). The H7N9 virus is thought to have emerged upon four reassortment events between H9N2 viruses and avian-origin viruses of subtype H7 and N9 (Lam et al, 2013). The H9N2 virus itself has been shown to have zoonotic potential as well,

resulting in relatively mild human infections upon contact with poultry. H9N2 viruses remain enzootic in domestic birds in many countries of the Eastern Hemisphere (Peiris et al, 1999).

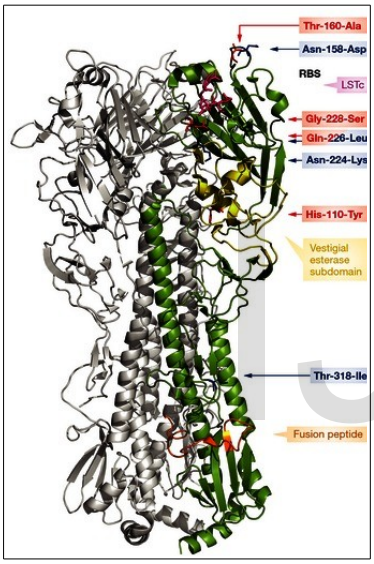
For all influenza pandemics of the last century, the animal-origin viruses acquired the ability to transmit efficiently via aerosols or respiratory droplets (hereafter referred to as “airborne transmission”) between humans (Sorrell et al, 2011). To evaluate airborne transmission in laboratory settings, ferret and guinea pig transmission models were developed, in which cages with uninfected recipient animals are placed adjacent to cages with infected donor animals. The experimental setup was designed to prevent direct contact or fomite transmission, but to allow airflow from the donor to the recipient ferret (Lowen et al, 2006; Maines et al, 2006). Using the ferret transmission model, pandemic and epidemic viruses isolated from humans are generally transmitted efficiently via the airborne route, whereas avian viruses are generally not airborne transmissible (Sorrell et al, 2011; Belser et al, 2013b).

Why some animal influenza viruses frequently infect humans, whereas others do not, and how viruses adapt to become airborne transmissible between mammals have been key questions in influenza virus research over the last decade. Studies on H1N1, H2N2, and H3N2 viruses from the 1918, 1957, and 1968 pandemics, respectively, revealed that interaction of HA with virus receptors on host cells was a critical determinant of host adaptation and airborne transmission between ferrets (Tumpey et al, 2007; Pappas et al, 2010; Roberts et al, 2011). Sorrell and colleagues showed that a wild-type avian H9N2 virus was not airborne transmissible in ferrets, but reassortment with a human H3N2 virus and subsequent adaptation in ferrets yielded an airborne transmissible H9N2 virus, primarily due to changes in the H9N2 HA and NA surface glycoproteins (Sorrell et al, 2009). Several laboratories have studied the transmissibility of avian H5N1 viruses and their potential to become airborne, and four studies recently described airborne transmission of laboratory-generated H5N1 influenza viruses. Three of these studies—two using ferrets and one using guinea pigs—used H5 reassortant viruses between human pH1N1 or H3N2 viruses and H5N1 virus (Chen et al, 2012; Imai et al, 2012; Zhang et al, 2013d). Herfst et al(2012)were the first to show mammalian adaptation of a fully avian H5N1 virus to yield an airborne transmissible virus in ferrets. Although avian H7 influenza viruses are generally not transmitted via the airborne route between ferrets (Belser et al, 2008), H7N9 strains from the Chinese 2013 outbreak were shown to be transmitted via the airborne route without further adaptation, albeit less efficiently as compared to human seasonal and pandemic viruses No transmission was observed from pigs to ferrets.

It has thus become increasingly clear that animal influenza viruses beyond the H1, H2, and H3 subtypes—specifically H5, H7, and H9—can acquire the ability of airborne transmission between mammals. To date, the exact genetic requirements for animal influenza virus to cross the species barrier and establish efficient human-to-human transmission remain largely unknown, but receptor binding specificity is clearly one of the key factors.

HA as determinant of receptor binding specificity

As a first step to entry and infection, influenza viruses attach with the HA protein to sialylated glycan receptors on host cells. The influenza virus HA protein is a type I integral membrane glycoprotein, with a N-terminal signal sequence. Post-translational modifications include glycosylation of the HA and acylation of the cytoplasmic tail region. Cleavage of the HA (HA0) by cellular proteases generates the HA1 and HA2 subunits, which form a disulfide bond-linked complex. The HA protein forms trimers, with each monomer containing a receptor binding site (RBS) capable of engaging a sialylated glycan receptor, a vestigial esterase subdomain, and a fusion subdomain



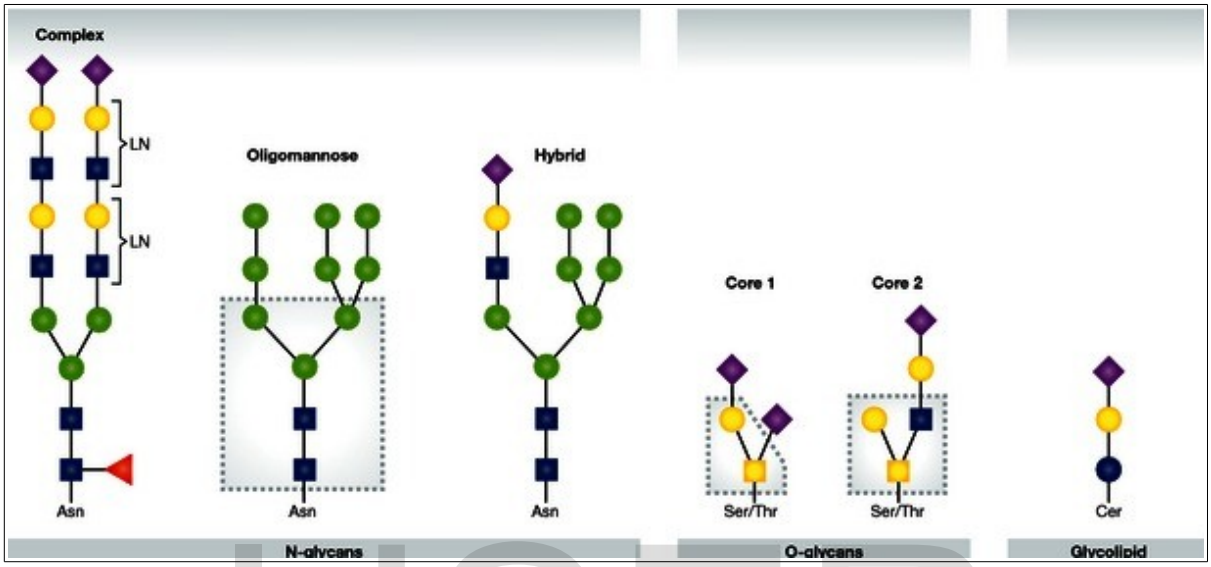
Influenza virus receptors

Types of glycan structures

Mammalian cells are covered by a glycocalyx, which consists of glycolipids, glycoproteins, glycophospholipid anchors and proteoglycans (Varki & Varki, 2007; Varki & Sharon, 2009). Glycans represent one of the fundamental building blocks of life and have essential roles in numerous physiological and pathological processes (reviewed in Hart, 2013). Glycans that are exposed on the exterior surface of cells play an important role in the attachment of toxins and pathogens like influenza A viruses.

Several types of glycans exist, including N-glycans, O-glycans, and glycolipids (reviewed in Brockhausen et al, 2009; Schnaar et al,2009; Stanley et al, 2009). The N-glycans can be further divided into different types such as “complex,” “hybrid,” and “oligomannose.” There is a wide

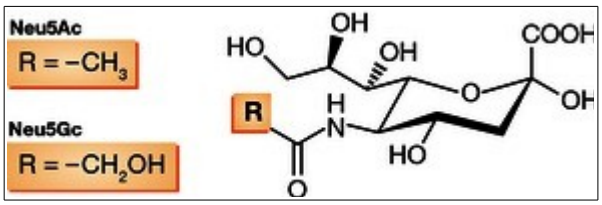
variety of O-glycans, with eight different core structures. Cores 1 and 2 are common structures that are present on glycoproteins. This particular classification of glycan structures is not necessarily relevant for binding of influenza viruses. For O-glycans and N-glycans, there is extensive structural diversification at the termini of glycan chains, which is potentially more important than differences in the core structures.

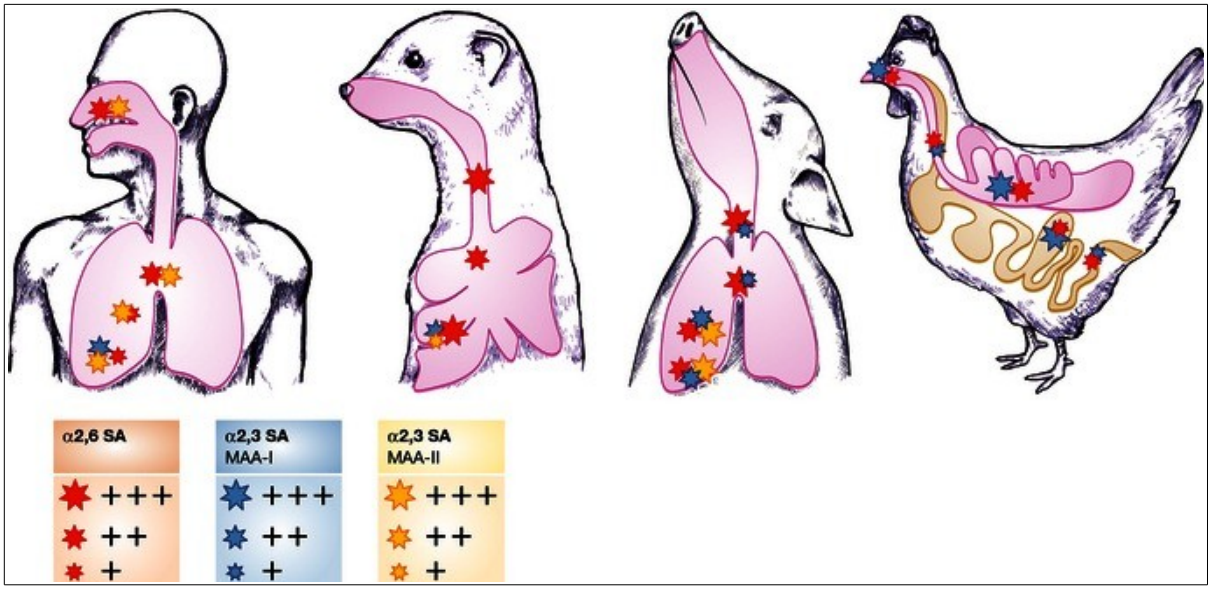


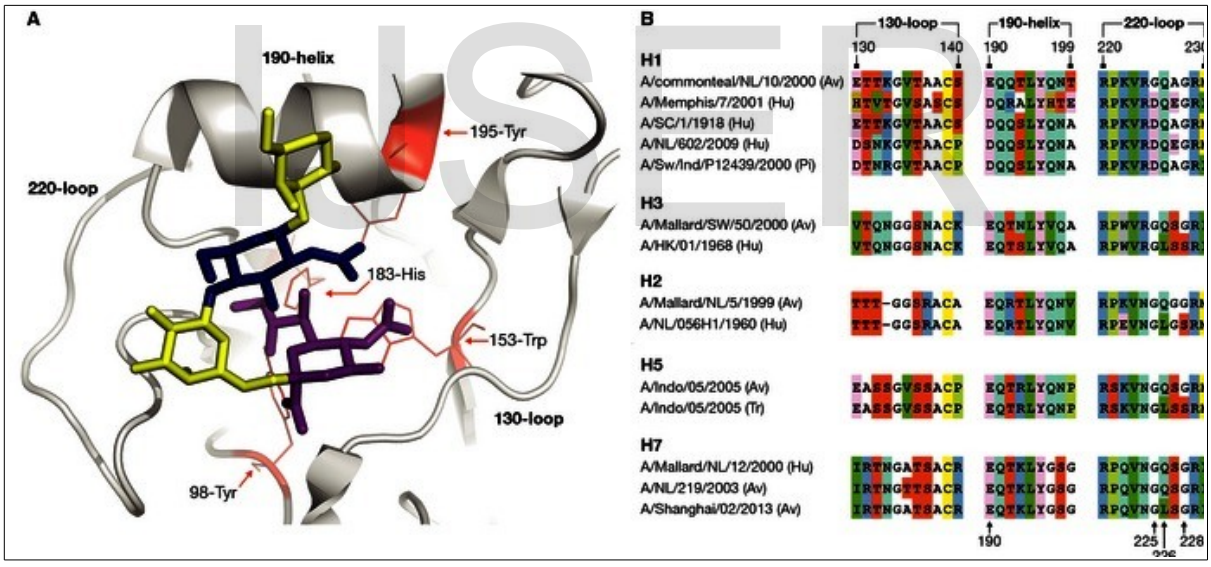
N-glycans, O-glycans, and glycolipids
Examples of three types of N-glycans are shown; complex with multiple N-acetyllactosamine repeats (LN), oligomannose, and hybrid (with the common core boxed). A glycolipid and two types of O-glycans are also shown; core 1 (in box) and core 2 (in box) O-glycans. Monosaccharides are depicted using the following symbolic representations: fucose (red triangle), galactose (yellow circle), N-acetyl glucosamine (blue square), N-acetyl galactosamine (yellow square), glucosamine (blue circle), mannose (green circle), sialic acid (purple diamond).

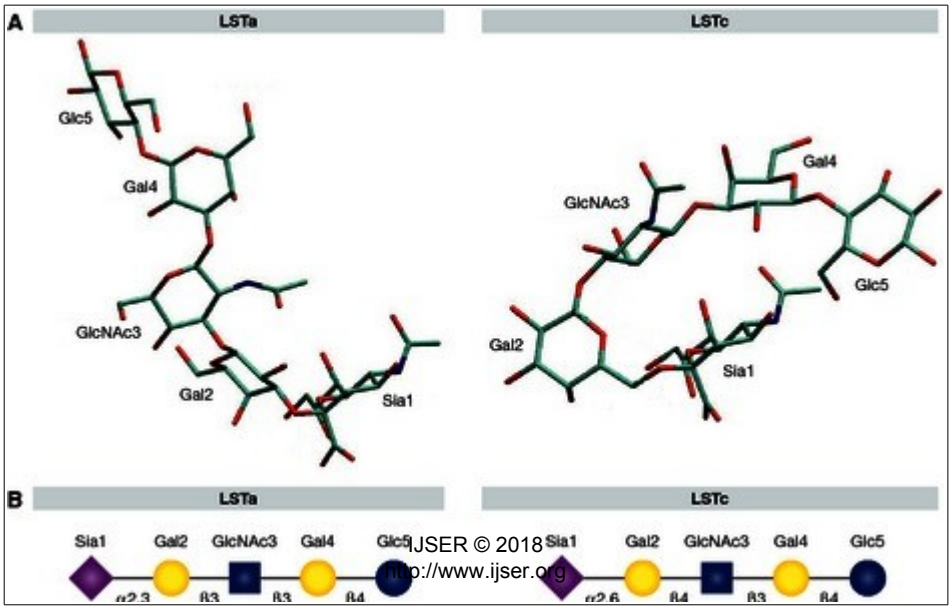
Types of sialic acid

SAs share a nine-carbon backbone and are among the most diverse sugars found on glycan chains of mammalian cell surfaces. Common SAs found in mammals are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc; reviewed in Varki & Varki, 2007). Neu5Gc has been detected in pigs, monkeys, and a number of bird species, but humans and some avian species like chickens or other poultry, do not contain Neu5Gc or only minor quantities (Chou et al, 1998; Muchmore et al, 1998; Schauer et al, 2009; Walther et al, 2013).




α2,6 SA
α2,3 SA
MAA-I
α2,3 SA
MAA-II
+++
++
+


A
190-helix
195-Tyr
220-loop
183-His
153-Trp
130-loop
98-Tyr
B
130-loop
190-helix
220-loop
H1
A/commonteal/NL/10/2000 (Av)
A/Memphis/7/2001 (Hu)
A/SC/1/1918 (Hu)
A/NL/602/2009 (Hu)
A/Sw/Ind/P12439/2000 (Pi)
H3
A/Mallard/SW/50/2000 (Av)
A/HK/01/1968 (Hu)
H2
A/Mallard/NL/5/1999 (Av)
A/NL/056H1/1960 (Hu)
H5
A/Indo/05/2005 (Av)
A/Indo/05/2005 (Tr)
H7
A/Mallard/NL/12/2000 (Hu)
A/NL/219/2003 (Av)
A/Shanghai/02/2013 (Av)

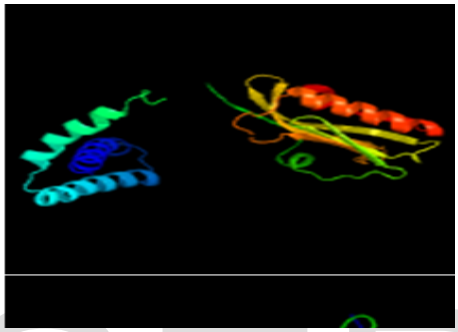

A
LSTa
LSTc
Glc5
Gal4
GlcNAc3
Gal2
Sia1
B
LSTa
LSTc
Sia1
Gal2
GlcNAc3
Gal4
Glc5
α2,3
β3
β3
β4
α2,6
β4
β3
β4

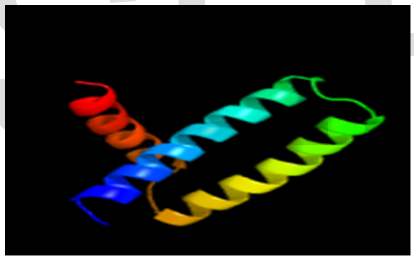
Principle:
The BLAST sequences are first analysed in sequence manipulation suite to find out the codon plot, CpG islands in order to track the receptors and the dock it with the 3F5T receptor

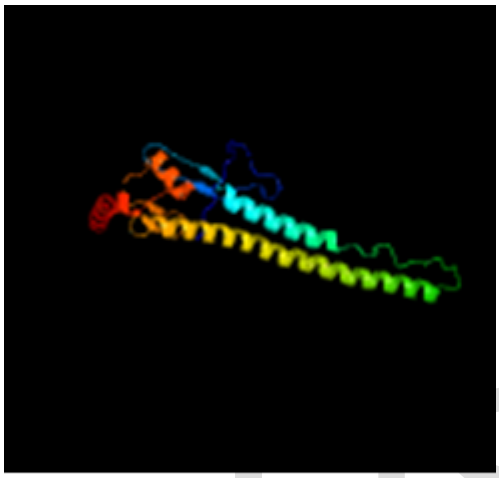
Results:-

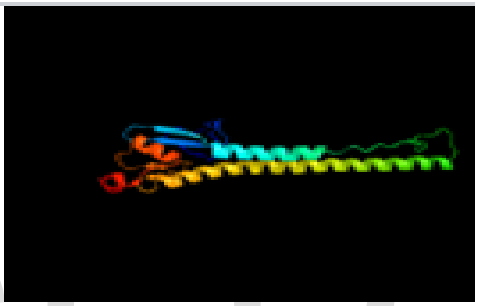
Below are the images of protein protein docking

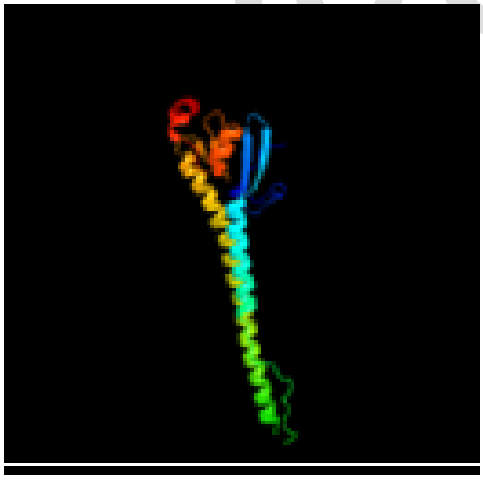
Secondary structure:



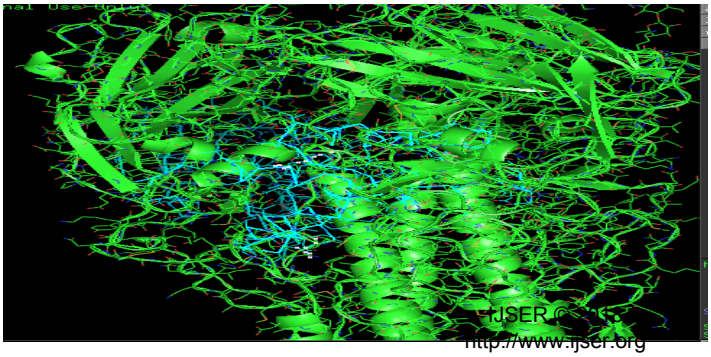








Tertiary structure



Due to the circulation of H5N1 virus strains such as HK212, HK213 and DKGX/35, these viruses can recognize both SAs in α-2,3Gal and α-2,6Gal linkages. In addition, certain amino acid substitutions in HA can switch the binding specificity of a virus from SAα-2,3Gal to SAα-2,6Gal, which can affect organ tropism and even enable transmission between ferrets. In contrast to H5N1 virus, the 2009 H1N1 pandemic virus preferentially binds to the SAα-2,6Gal receptor although viruses with a D222G mutation in HA switch the viral receptor binding preference from SAα-2,6Gal to SAα-2,3Gal and were frequently observed in the lower respiratory tracts of patients with severe clinical outcomes. Therefore, the binding properties of influenza virus HA to glycan receptors affect interspecies transmission, organ tropism and virulence in the host. Thus, when regions with H5N1 in circulation are continually surveyed, it should be noted if amino acid mutations exist that are related to binding affinity, as this may provide early evidence for the genesis of a pandemic virus and should contribute to future pandemic prevention efforts.