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ORIGINAL PAPER

# Molecular modeling, docking and dynamics simulations of GNA-related lectins for potential prevention of influenza virus (H1N1)

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**Abstract** The *Galanthus nivalis* agglutinin (GNA)-related lectin family exhibit significant anti-HIV and anti-HSV properties that are closely related to their carbohydrate-binding activities. However, there is still no conclusive evidence that GNA-related lectins possess anti-influenza properties. The hemagglutinin (HA) of influenza virus is a surface protein that is involved in binding host cell sialic acid during the early stages of infection. Herein, we studied the 3D-QSARs (three-dimensional quantitative structure–activity relationships) of lectin– and HA–sialic acid by molecular modeling. The affinities and stabilities of lectin– and HA–sialic acid complexes were also assessed by molecular docking and molecular dynamics simulations. Finally, anti-influenza GNA-related lectins that possess stable conformations and higher binding affinities for sialic acid than HAs of human influenza virus were screened, and a possible mechanism was proposed. Accordingly, our results indicate that some GNA-related lectins, such as *Yucca filamentosa* lectin and *Polygonatum cyrtonema* lectin, could act as drugs that prevent influenza virus infection via competitive binding. In conclusion, the GNA-related lectin family may be helpful in the design of novel candidate agents for preventing influenza A infection through the use of competitive combination against sialic acid specific viral infection.

Huai-long Xu and Chun-yang Li contributed equally to this work

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**Keywords** *Galanthus nivalis* agglutinin (GNA)-related lectins · Hemagglutinin (HA) · Influenza A virus · *Polygonatum cyrtonema* lectin (PCL) · Viral infection

**Abbreviations**

| | |
|---|---|
| GNA | *Galanthus nivalis* agglutinin |
| TGL | *Tulipa gesneriana* lectin |
| YFL-II | *Yucca filamentosa* lectin |
| YFL-I | *Yucca filamentosa* lectin |
| 3D-QSAR | Three-dimensional quantitative structure–activity relationship |
| AML[a] | *Arum maculatum* lectin |
| AAL | *Arisaema amurense* lectin |
| PLC | *Pinellia cordata* lectin |
| AML[b] | *Alocasia macrorrhiza* lectin |
| PTL | *Pinellia ternata* lectin |
| PCL | *Polygonatum cyrtonema* lectin |
| AHL | *Arisaema heterophyllum* lectin |
| HA | Hemagglutinin |
| HA-I | 1934 Human H1 HA |
| HA-II | 1918 Human H1 HA |
| vRNP | Viral nucleoprotein |

## Introduction

Plant lectins are an important type of glycoprotein that are widespread throughout the plant kingdom and possess the ability to recognize carbohydrate moieties specifically as well as to bind with carbohydrates reversibly [1–3]. The *Galanthus nivalis* agglutinin (GNA)-related lectin family, a significant family of plant lectins, has drawn increasing attention in recent years due to its remarkable antitumor and antiviral properties. These notable biological properties of the GNA-

related lectin family are closely related to their carbohydrate-binding activities [4–6]. Most GNA-related lectins exhibit mannose-binding activity, while several lectins such as PCL (*Polygonatum cyrtonema* lectin) [7] and OJL (*Ophiopogon japonicus* lectin) [8] also display carbohydrate affinity towards sialic acid. Several GNA-related lectins exhibit significant antiviral properties, such as the anti-HIV activity of GNA [4] and PCL [9] as well as the anti-HSV-II effect of OJL [8, 10]. Whether GNA-related lectins can also show similar anti-influenza properties remains to be verified.

The surface of the influenza A virus is made up of diverse proteins; among these, the most important envelope glycoprotein implicated in receptor recognition and fusion is hemagglutinin (HA). Another essential glycoprotein is neuraminidase (NA), which assists HA in binding with the corresponding receptor and initiating infection [11]. HA exhibits a variety of distinct structures, and all of these different types of HA can bind receptors containing glycans with terminal sialic acids [11–13]. The combination of HA with sialylated glycans exposed on the host cell surface is the key first step in the infection, transmission and virulence of influenza viruses [14, 15]. Although HA must accumulate mutations rapidly and continuously to escape immune system recognition, one recent report verified that HA of the 2009 influenza A virus was extremely similar to the HA of the 1918 influenza A virus [16]. As the influenza A virus has mutated, it has infected species ranging from avians to humans; avians are mainly infected by α2,3-linked sialic acid specific influenza A virus strains while humans are mainly infected by α2,6-linked sialic acid specific influenza A virus strains [17–21].

In the current study, we modeled the three-dimensional structures of GNA-related lectins and assessed the affinities and stabilities of lectin– and HA–sialic acid complexes via molecular docking and dynamics simulations. In addition, we screened potential GNA-related lectins against human influenza virus, and proposed possible mechanisms for the activity of such lectins toward human influenza virus. Accordingly, our results indicate that some GNA-related lectins would be promising candidate agents for preventing influenza virus infections, as they would competitively bind with receptors containing glycans with terminal sialic acids. Thus, these findings may lead to the exploration of the GNA-related lectin family as potential agents against various sialic acid specific viral infections in the future.

## Materials and methods

### Data preparation

The structures of two representative HAs were retrieved from the Protein Data Bank (PDB) (http://www.pdb.org/pdb/home/home.do); the PDB codes were 1RU7 (1934 Human H1 Hemagglutinin) and 1RUZ (1918 H1 Hemagglutinin), respectively. The sequences of GNA-related lectins were acquired from Universal Protein Resource (http://www.uniprot.org) and one study performed by Els [22]. All of the sequences were further screened, and finally 51 potential sialic acid binding GNA-related lectins were obtained. The sialic acid structure was separated from HA of the 1934 influenza A virus in complex with a known human receptor analog, LSTc (PDB ID: 1RVZ), using the Chimera program [23].

### Molecular modeling and structural alignment

Domains of these lectin sequences were acquired by Prosite (http://www.expasy.ch/prosite/) [24], and these domains were further utilized to construct the three-dimensional structures of 51 GNA-related lectins using MODELLER9v7 software [25], with the structure of GNA (*Galanthus nivalis*) lectin, PDB ID: 1JPC [26]), used as the template. All structures were optimized using the Chimera program; optimization included hydrogen atom additions and energy minimizations, as well as the elimination of bound crystal water molecules or other organic compounds [23]. The Chimera MatchMaker tool [23] was utilized to compare the homology modeling structure and the crystal structure of PCL retrieved from the Protein Data Bank (PDB ID: 3A0C [39]).

### Docking experiment

All molecular docking calculations were performed using the UCSF DOCK6.3 program [27] with a flexible ligand docking to a rigid receptor in a grid-based scoring method. The flexible ligand docking algorithm in DOCK6.3 allows the ligand (sialic acid) to structurally rearrange in response to the receptor (GNA-related lectin and HA) using the anchor-and-grow feature, which first identifies the largest rigid substructure (the anchor) and the flexible layers of the ligand, and then builds the flexible layers of the ligand onto the best anchor orientations within the context of the receptor (the growth stage). In our docking process, we increased the maximum number of orientations to 1000.

### Molecular dynamics (MD) simulations

Molecular dynamics simulations were performed using the GROMACS (version 4.0.5) software package [28], with the GROMOS96 43a2 force-field [29] and a single-point-charge (spc) water model [30]. The sialic acid topology was generated using the Dundee PRODRG2 server [31], and the simulations of HA– and lectin–sialic acid complexes were performed under the same conditions.

Long-range electrostatic interactions were computed by a particle mesh Ewald (PME) algorithm at every step [32,

33]. The linear constraint (LINCS) [34] and Berendsen coupling [35] algorithms were utilized to constrain the lengths of all bonds and to keep the pressure (1 bar) and temperature (300 K) constant, so that a time step of 2 fs could be employed under isothermal–isobaric conditions. The cutoff distance used for van der Waals (VdW) forces was 1.4 nm, and the coordinates were saved every 500 steps. The resulting trajectory files were viewed and analyzed using VMD software [36].

## Results

### Overall structures of GNA-related lectins and hemagglutinin

Most GNA-related lectins are mannose specific, and the significant conserved "QXDXNXVXY" motif of the subdomain plays a crucial role in mannose recognition [8, 37, 38]. However, several lectins also exhibit sialic acid binding activity that may result from an amino acid mutation in the conservative mannose-binding motif of GNA-related lectins. Therefore, all of the sequences were further screened, and 51 GNA-related lectins (as shown in Fig. S1 of the "Electronic supplementary material," ESM) possessing potential sialic acid specificity were finally obtained.

It has been reported that the monomers of GNA-related lectins have similar three-dimensional structures containing three subdomains. Each subdomain is made up of three or four strands of antiparallel β-sheets connected by Ω-loops. All of the 51 lectins exhibited similar three-dimensional structures (data not shown), and possessed sialic acid binding activity. For example, in YFL-I (*Yucca filamentosa* lectin) and AML[a] (*Arum maculatum* lectin), the three-dimensional structures of YFL-I and AML[a] (see Fig. 1a and c) display similar conformations. The motions of the YFL-I–Sia complex are also shown in Video S1 of the ESM.

To further validate the abovementioned results, we retrieved the crystal structure (determined by X-ray diffraction; PDB code: 3A0C) of the PCL with anti-HIV and mannose-binding activities [39]. Figure 2 clearly shows that the PCL structure obtained by homology modeling bears a close resemblance to the three-dimensional structure of PCL obtained by X-ray diffraction, with a RMSD in the Cα position of 0.680 Å. This comparison indicates that the structure from homology modeling is very similar to the native crystal structure, which supports the hypothesis mentioned above.

In addition, sialic acid binding sites of receptor HA consisted mainly of the 190 helix (residues 190 to 198), the 130 loop (residues 135 to 138) and the 220 loop (residues 221–228). Our docking results illustrated that sialic acid could bind to these sites, with the HA-I (1934 Human H1 HA)–Sia complex forming four hydrogen bonds involving His180, Glu212, Asn227 and Tyr229 (Table 1 and Fig. 1e). The HA-II (1918 Human H1 HA) system formed three hydrogen bonds involving Glu213, Arg217 and Arg226 (Table 1 and Fig. 1f). HA-I in complex with sialic acid is visualized in the video S2 in the ESM.

### Binding energies

Accordingly, we docked two HAs of human influenza virus [PDB codes: 1RU7 (HA-I) and 1RUZ (HA-II)], as well as 51 GNA-related lectins with sialic acid. Ten GNA-related lectins showed greater affinities for sialic acid than the HAs, as shown in Table 1 (these structures are shown in Fig. 1 and Fig. S2 of the ESM). The binding energies of these ten GNA-related lectins in complex with sialic acid range from −41.15 kcal $mol^{-1}$ to −46.55 kcal $mol^{-1}$, although *Tulipa gesneriana* lectin (TGL) possesses the highest specificity for sialic acid. The binding energies of the two HA–Sia complexes were −40.84 kcal $mol^{-1}$ and −40.85 kcal $mol^{-1}$, respectively. Consistent with the molecular dynamics simulation results, the average total energies of the lectin– and HA–Sia complexes also indicated that the specificities of the two HAs for sialic acid were similar, and that TGL possessed the highest affinity for sialic acid (as shown in Table 1).

Further investigations of the overall structures of GNA-related lectin and sialic acid conjugates demonstrated that there were two distinct binding types of sialic acid, which meant that the GNA-related lectins could be largely divided into two groups. For instance, TGL– and YFL–Sia belonged to the first binding type, where the sialic acid binds to the center of the lectin (Fig. 1a, b and Fig. S2 of the ESM). Other complexes such as AML[a]–Sia, *Arisaema amurense* lectin (AAL)–Sia, and *Polygonatum cyrtonema* lectin (PCL)–Sia belong to the second binding type, where sialic acid is almost bound to the second subdomain of the GNA-related lectin (Fig. 1c, d and Fig. S2 of the ESM).

Exploration of the binding sites of the GNA-related lectin–Sia complexes showed that the second conserved motif of "QXDXNXVXY" was mainly implicated in sialic acid recognition; in this motif, the majority of the key residues were K, E and K, which had mutated from the conserved D, N and Y residues, respectively. The R or T residues of several GNA-related lectins may also be involved in sialic acid binding (Table 1 and Fig. 3).

### Root mean square deviations

To evaluate the stabilities of lectin– and HA–Sia complexes during the MD simulations, we calculated the root mean square deviation (RMSD) with respect to the initial structures along the 1.2 ns trajectory (shown in Fig. 4). These results indicated that the first binding type of lectin–

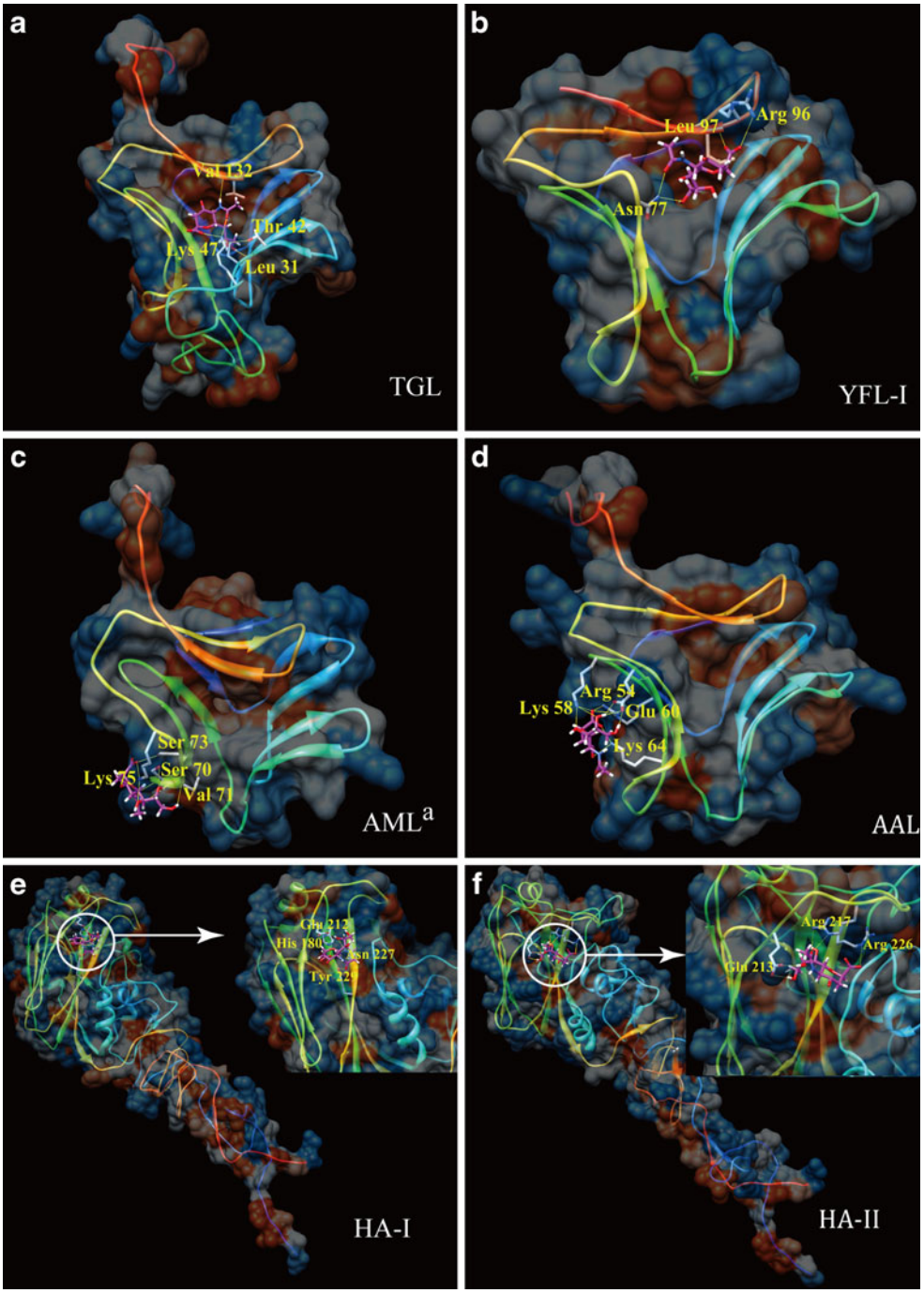



**Fig. 1** The overall modeling of protein (GNA-related lectins and HAs)–sialic acid complexes: TGL–sialic acid (**a**), YFL-I–sialic acid (**b**), AML[a]–sialic acid (**c**), AAL–sialic acid (**d**), 1934 Human H1 HA–sialic acid (**e**), and 1918 Human H1 HA–sialic acid (**f**). All complexes are presented in surface potential mode and stickball mode. In stickball mode, the sialic acid is represented by a *purple stick*, while the hydrogen bonds are shown as *yellow lines*

Sia complexes could reach equilibration and oscillate around an average value. YFL-I possessed the lowest RMSD and reached the plateau value after about 200 ps of simulation time. YFL-II reached its plateau value at a similar time, but the conformation of the YFL-II–Sia system was found to be less stable than that of YFL-I, whereas the TGL–Sia system showed more variability.

In addition, RMSD analyses of the second binding type of lectin–Sia complexes illustrated that three [AHL (*Arisaema heterophyllum* lectin), AML[b] (*Alocasia macrorrhiza* lectin) and PCL] of the seven GNA-related lectins equilibrated quickly after about 550 ps of simulation time, while the other four complexes were more variable. AHL was able to reach its plateau value of about 0.45 nm after 250 ps simulation time, and it maintained a stable conformation. Figure 4c indicates that AHL–Sia is more stable than the corresponding complexes of the other six lectins, despite the fact that its RMSD value was not the lowest. However,

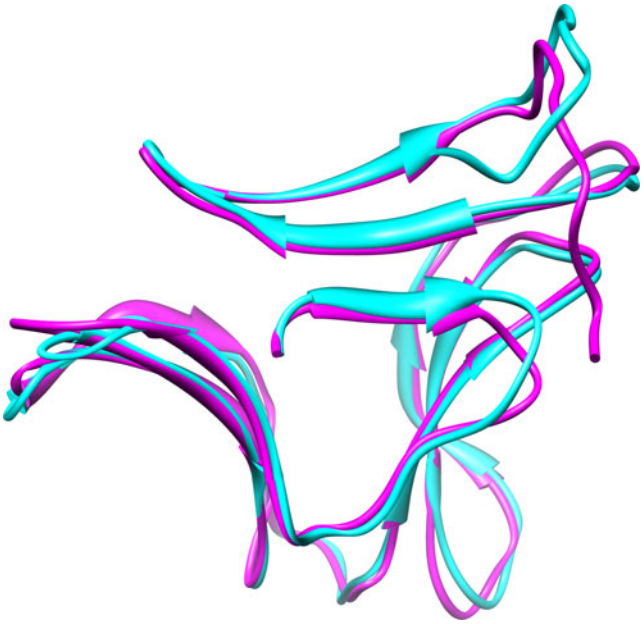


**Fig. 2** Structural similarity between the structure obtained from homology modeling and the crystal structure of PCL. The structure of PCL from homology modeling is colored *cyan*, while its crystal structure is colored *magenta*

the RMSD results for HA–sialic acid complexes demonstrated that they were more variable during the MD simulations. These results indicate that lectin–Sia complexes are more stable than HA–Sia complexes.

## The secondary structure

To further gauge the stabilities of lectin– and HA–Sia complexes, the variation in the secondary structure was analyzed across 1.2 ns trajectories (as shown in Fig. 5 and Fig. S3 of the ESM). The results clearly show that the conformations of lectin– and HA–Sia remained stable during the 1.2 ns MD simulations. As mentioned above, GNA-related lectins are made up of three or four antiparallel β-sheets connected by Ω-loops. Secondary structural analyses also showed that there were several slight variations in the lectin–Sia conformations during MD simulations. For instance, residues 78–82 of PCL (the β-sheet) changed from a coil to a β-sheet during the MD simulations. However, lectin–Sia binding domains, such as residues 96–97 of YFL-I and residues 70–75 of AML[a], were relatively stable during MD simulation.

Although HA–Sia complexes and binding domains between HAs and sialic acid also remained relatively stable during the 1.2 ns MD simulations, several slight variations disturbed the stabilities of these two systems, such as those associated with residues 210–229 of HA-I (shown in Fig. 5 and Fig. S3 of the ESM).

## Hydrogen-bonding analysis

As it is one of the most important forces that maintains the binding between ligand and receptor, hydrogen bonding can also reflect complex stability. Thus, we analyzed the changes in the hydrogen bonding in the the binding domain between proteins (HAs or lectins) and sialic acid (shown in Fig. 6 and Fig. S4 of the ESM).

HA-I in complex with sialic acid reached equilibrium at 480–1200 ps, and the average number of hydrogen bonds between HA-I and sialic acid was 1.52. Although complexes of lectins such as AML[a] and PLC (*Pinellia cordata* lectin) with sialic acid presented more hydrogen bonds than

**Table 1** Results of molecular docking and molecular dynamics simulations of GNA-related lectins and HA in complex with sialic acid

| Protein name | Organism | Binding energy[a] | Total energy[b] | Hydrogen bonds[a] | Binding type |
|---|---|---|---|---|---|
| TGL | *Tulipa gesneriana* | −46.55 | −363977 | Lys47, Val132, Thr42, Leu31 | I |
| YFL-II | *Yucca filamentosa* | −44.10 | −162093 | Lys81, Leu103 | I |
| YFL-I | *Yucca filamentosa* | −43.92 | −225707 | Asn77,Arg96, Leu97 | I |
| AML[a] | *Arum maculatum* (Cuckoo-pint) | −43.95 | −285397 | Lys75, Ser73, Ser70, Val71 | II |
| AAL | *Arisaema amurense* | −43.90 | −327434 | Glu60, Lys58, Arg54, Lys64 | II |
| PLC | *Pinellia cordata* | −42.52 | −279799 | Asp55, Thr54, Thr52, Arg56, Lys62 | II |
| AML[b] | *Alocasia macrorrhiza* (Giant taro) | −41.86 | −228785 | Lys64, Asn56, Lys58, Glu60 | II |
| PTL | *Pinellia ternata* | −41.42 | −264601 | Glu60, Lys64 | II |
| PCL | *Polygonatum cyrtonema* | −41.27 | −187606 | Arg54, Thr56, His58, Asn59 | II |
| AHL | *Arisaema heterophyllum* | −41.15 | −239089 | Glu58, Lys62, Arg66 | II |
| HA-I | H1N1 | −40.84 | $-2.49\times10^{6}$ | His 180, Glu 212, Asn 227, Tyr 229 | – |
| HA-II | H1N1 | −40.85 | $-2.72\times10^{6}$ | Glu 213, Arg 217, Arg 226 | – |

[a] indicates molecular docking results

[b] indicates molecular dynamics simulation results

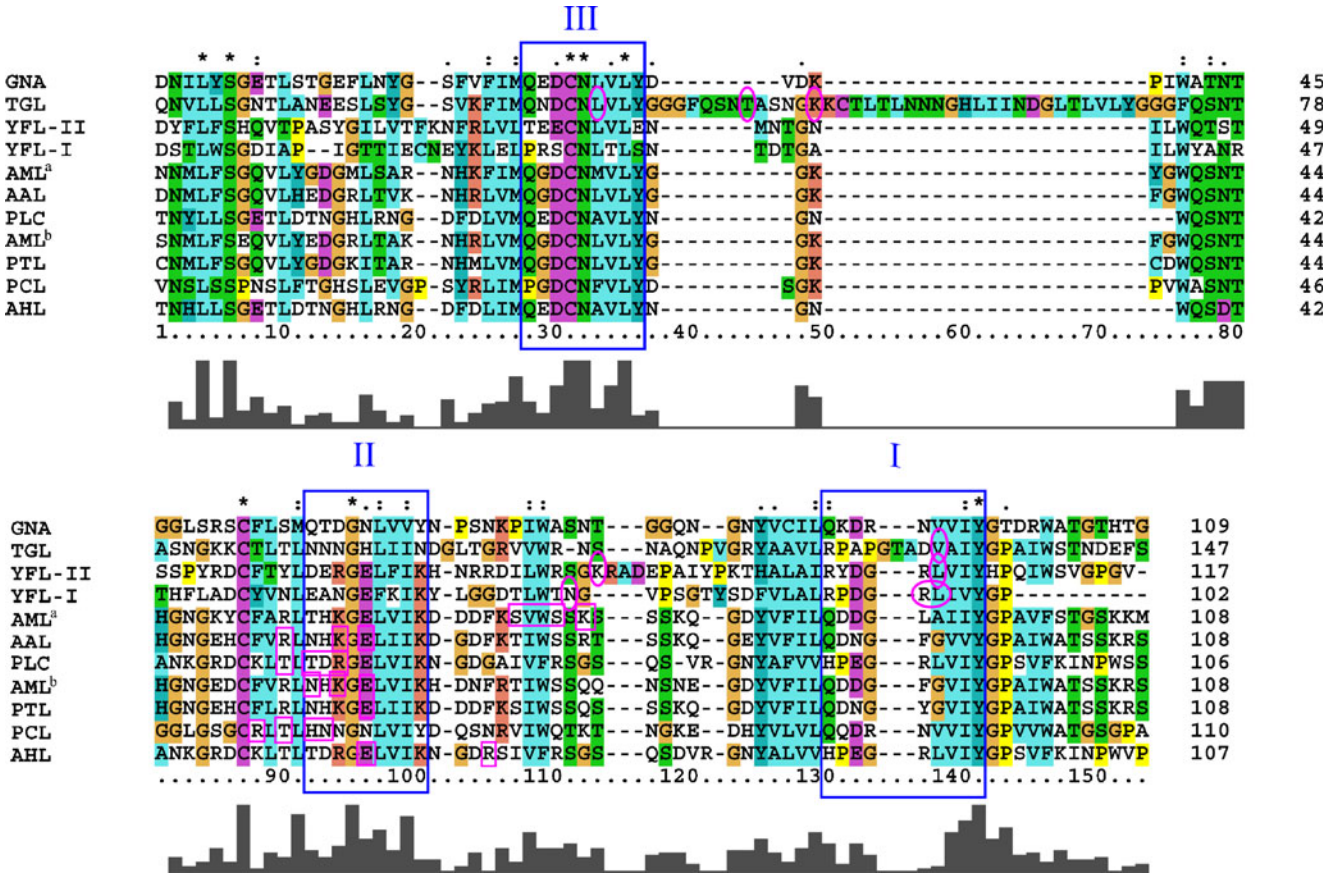



**Fig. 3** Sequence alignment of GNA with ten GNA-related lectins that exhibit significantly greater affinity for sialic acid than HA. GNA-related lectins show significant sequence similarities, and the second conserved motif is mainly implicated in sialic acid recognition

HA complexed with sialic acid (as shown in Fig. 6d), the conformations of AML[a] and PLC in complex with sialic acid tended to change more, according to RMSD analyses.

The RMSD analyses also showed that the average number of hydrogen bonds in the YFL-I–Sia complex was similar to the number of H-bonds in the HA–Sia system (as shown in Fig. 6b, d), but YFL-I exhibited a more stable structure. All of these results suggest that YFL-I may show more affinity than HA towards sialic acid.

## Discussion

The GNA-related lectin family shows structural conservation and is mannose specific. The significantly conserved "QXDXNXVXY" motif of the subdomain plays a crucial role in mannose recognition [8, 37, 38]. In addition to their affinity to mannose, several GNA-related lectins can also bind with sialic acid, which may be due to the mutation of the conservative mannose-binding motif [9]. These affinities for mannose and sialic acid are also closely related to the antitumor and antiviral properties of GNA-related lectins [4, 8].

The surface glycoprotein hemagglutinin (HA) of influenza A virus is specific to sialic acid, so it may infect the host by binding with receptors that contain glycans with terminal sialic acids [11–13]. Therefore, it is urgently necessary to study the competitive combination of GNA-related lectins and sialic acid with view to its potential antivirus activity, which mainly occurs through the mechanism of steric hindrance. The experiments described in this paper were based on a combined approach involving molecular modeling, docking and molecular dynamics screening of potential GNA-related lectins [40–42] that possess stable conformations when in complex with sialic acid, and higher affinities than HAs for sialic acid.

In our study, ten GNA-related lectins were found to have higher affinities than HAs for sialic acid, and the TGL–Sia and YFL-I–sialic acid complexes exhibited relatively strong binding activities according to molecular docking results. Consistent with these docking results, the average total complex energy—which is a good indicator of the stability of the system—suggested that TGL and YFL-I show the greatest specificities for sialic acid.

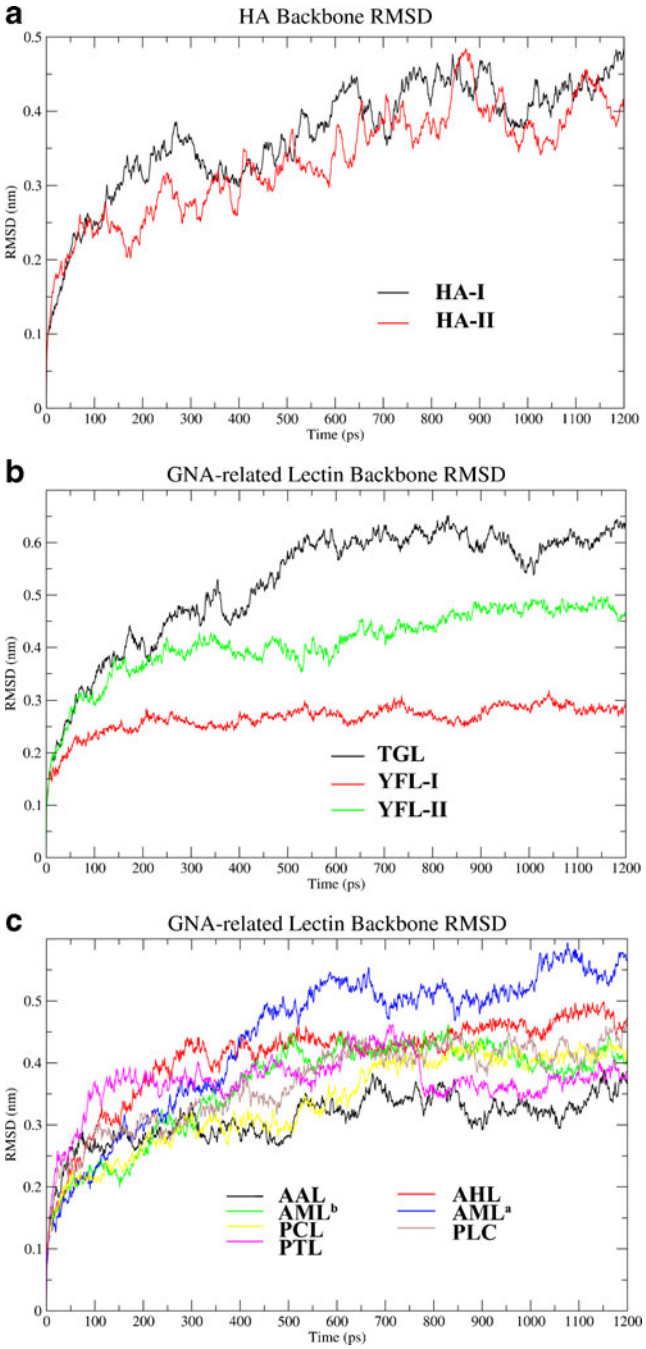



**Fig. 4** Root mean square deviations of protein (GNA-related lectins and HAs)–sialic acid complexes over time from molecular dynamics simulations for HA–sialic acid complexes (**a**), the first binding type of GNA-related lectin–sialic acid complexes (**b**), and the second binding type of GNA-related lectin–sialic acid complexes (**c**)

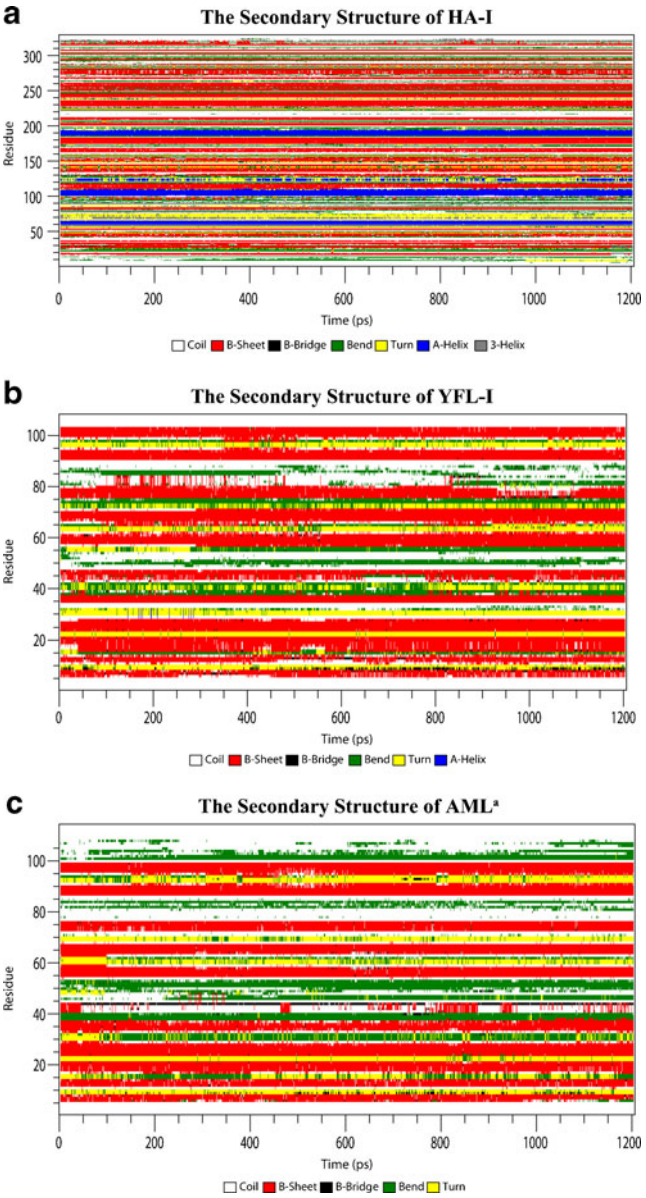
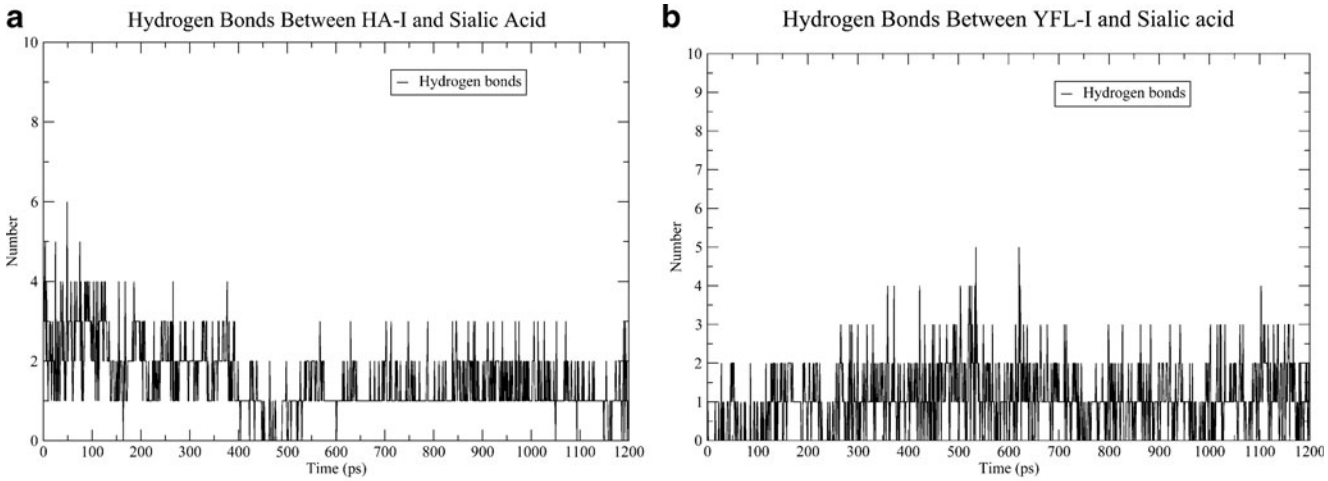



**Fig. 5** Secondary structural variations of protein (GNA-related lectins and HAs)–sialic acid complexes in molecular dynamics simulations. Secondary structural variations over time for: 1934 Human H1 HA–sialic acid (**a**), YFL-I–sialic acid (**b**) and AML[a]–sialic acid (**c**) complexes

Moreover, we assessed the stability of each of the ten systems by analyzing the root mean square deviation (RMSD), which indicates the Euclidean distance from the structure to a reference structure like the crystal structure or backbone, and only energy-stable complexes were accepted. RMSD results showed that most of the lectin–Sia complexes maintained a stable conformation throughout the 1.2 ns trajectory, while the YFL-I system exhibited the greatest stability. Besides the RMSD, secondary structural and hydrogen-bonding analyses can also depict the stability of such a system. In accord with the RMSD analyses, most of the lectin–sialic acid complexes exhibited relatively stable conformations throughout the simulations, although the YFL-I complex possessed the greatest stability.

If the lectin–Sia structure is stable and presents a greater affinity than HA for sialic acid, this GNA-related lectin could be screened for potential anti-H1N1 activity due to competitive binding. YFL-I–Sia complex seemed to be the least stable structure according to stability analyses, but




d

Average Hydrogen Bonds Between HA/Lectin and Sialic Acid During the MD Simulations

| Name | Hydrogen Bonds |
|---|---|
| HA-I | 1.52 |
| HA-II | 0.87 |
| TGL | 1.24 |
| YFL-I | 1.05 |
| YFL-II | 0.41 |
| AAL | 1.01 |
| AHL | 0.38 |
| AML[b] | 0.83 |
| AML[a] | 2.67 |
| PCL | 1.15 |
| PLC | 1.55 |
| PTL | 1.00 |

**Fig. 6** Hydrogen-bonding variations in protein (GNA-related lectins and HAs)–sialic acid complexes during molecular dynamics simulations: 1934 Human H1 HA–sialic acid (**a**), YFL-I–sialic acid (**b**) and AML[a]–sialic acid (**c**) complexes. **d** Average number of hydrogen bonds between HAs/lectins and sialic acid during the course of the simulation

YFL-I possessed a relatively strong affinity for sialic acid. TGL in complex with sialic acid presents the strongest specificity, while this system is not the most stable. These differences between the complexes may arise from different binding domains and overall structures. The reason that the TGL–Sia complex had the strongest affinity but not the most stable structure may be the long sequence length of TGL, which might lead to the generation of novel and different overall structures. The complex with AML[a] possessed more hydrogen bonds than that with YFL-I, which may be due to amino acid mutations in the carbohydrate recognition motif. In addition, structural uncertainties and inaccuracies of the docking and MD simulations could lead to these different results.

As mentioned above, YFL-I was found to have the greatest potential for use as a novel anti-influenza drug owing to the stable YFL-I–Sia conformation and the relatively strong affinity of YFL-I in complex with sialic acid. Accordingly, members of the GNA-related lectin family, which show similar properties to YFL-I, may be candidate drugs to fight influenza.

Recent reports have demonstrated that several GNA-related lectins possess notable antiviral activities; for example, the anti-HIV activities of GNA [4] and PCL [9], as well as the anti-HSV-II effect of OJL [8, 10]. GNA and PCL exert their anti-HIV activities by blocking the binding between HIV envelope glycoprotein gp120 and the corresponding receptor CD4 [4, 43]. The glycans of gp120 consist of a high-mannose type and a complex sialic acid type, and GNA-related lectins can bind with gp120 intensively, mainly due to their affinity for mannose and/or sialic acid [9, 10, 43, 44]. Accordingly, the combination of gp120 with GNA-related lectins instead of the receptor CD4 residing in host cells results in steric hindrance and prevents fusion and infection with HIV [4, 9, 43].

Similar to the anti-HIV mechanism of GNA-related lectins, we speculate that the binding between GNA-related lectins and sialic acid exposed on the glycoprotein receptors of host cells would prevent influenza A virus infection, mainly by blocking the combination of virus envelope glycoprotein HA with its corresponding sialic acid-linked receptor. Thus, GNA-related lectins that possess affinity for sialic acid would exert anti-influenza effects.

The antivirus activities of GNA-related lectins are realized by preventing the recognition between sialic acid and HA [45–47], whereas zanamivir and oseltamivir (two anti-H5N1 drugs) act mainly through the inhibition of the sialidase (also known as NA) activity of influenza A virus. NA plays a crucial role in cleaving sialic acid residues, and assists the release of progeny virions from infected cells [46]. Thus, the inhibition of sialidase activity could prevent progeny virion release, suppressing influenza A virus infection.

Some reports have revealed that HAs must accumulate mutations rapidly and continuously to escape recognition by the immune system [48, 49]. As the influenza A virus has mutated, it has affected a range of species from avians to humans. Avians are mainly infected by α2,3-linked sialic acid specific influenza A virus strains, while humans are mainly infected by α2,6-linked sialic acid specific influenza A virus strains [46, 50]. It has been suggested that the sialic acid affinity of HA may have remained unchanged during the evolution of this virus, despite the mutation of the HAs. If this is true, the anti-influenza activities of GNA-related lectins should be efficient and broad-spectrum.

In addition, GNA-related lectins may also exhibit the ability to selectively inhibit virus signaling pathways. As demonstrated in previous studies, the Ras-Raf and PI3K-Akt signaling pathways are the two of the most significant regulation mechanisms in virus propagation and vRNP (viral nucleoprotein) export [51–53]. The Ras-Raf signaling pathway appears to be particularly beneficial to virus replication, while blocking it results in strongly impaired growth of the influenza A virus [52, 53]. Additionally, the PI3K-Akt signaling pathway (considered to be a two-faced participant in virus defense and injection)—in contrast to most other antiviral signaling events—is not suppressed but rather activated in the presence of the viral NS1 protein at a very early stage in the replication cycle [52, 54, 55]. Nevertheless, recent research provides rational evidence that some GNA-related lectins, especially PCL, could induce murine fibrosarcoma L929 cell apoptosis and autophagy by negatively regulating the Ras-Raf and PI3K-Akt signaling pathways [56]. Here, GNA-related lectins could obviously act as antagonists in intracellular signaling pathways, inducing apoptosis and/or autophagy of cells infected with influenza A by blocking the Ras-Raf and PI3K-Akt pathways. However, more genomics and proteomics studies investigating this potential mechanism of GNA-related lectins are needed to support this viewpoint.

## Conclusions

In summary, our results demonstrate that YFL-I appears to have the most potential as an anti-influenza agent due to its relatively strong affinity for sialic acid and its stable conformation when in complex with sialic acid. Other GNA-related lectins with similar properties to YFL-I may also exert anti-influenza activities by competitively blocking the combination of H1N1 virus envelope glycoprotein HA with its corresponding sialic acid-linked receptor in the host cell. Elucidating the anti-influenza effects of GNA-related lectins would lead to deeper investigations of the influenza A virus and other viruses that show sialic acid specificity, which would in turn allow potential candidate agents from the GNA-related lectin family against various sialic acid specific virus infections to be identified.

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