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Computational Interrogation of The Human Norovirus-Host Cell Interactions Facilitated by A-Type Antigen

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Abstract

Norovirus infectivity, which causes norovirus-induced gastroenteritis, depends on the interaction between capsid protein VP1 of the virus and host cell HGBA receptors that tailor the cell membrane surface. The interaction results in VP1-HGBA complex formation prior to infection. The details of this interaction have been provided by x-ray structures of HGBA-VP1 complexes, but the dynamic nature of this interaction is not fully uncovered. Therefore, the dynamics that drive the formation of VP1-HGBA complex, which is crucial for developing new therapeutic approaches to find a cure for gastroenteritis disease, need to be elucidated. Here, we computationally analyzed the wild type VP1 capsid protein in complex with A-type HGBA antigen to unravel interactions that are important for virus to enter inside the host cell during infection. We have found that the ligand binding causes a fluctuation in a distant loop which resides in the interface of capsid building blocks, VP proteins. This fluctuation leads an instability in capsid particle that may be an indication for virus uncoating mechanism during the cell penetration.

Keywords

Norovirus, therapeutic antibody, HBGA blockage, molecular dynamics

1. INTRODUCTION

Norovirus-induced gastroenteritis, which was named hyperemesis hiemis earlier, was first discovered by Dr. John Zahorsky in 1929. It took almost 50 years, until 1972 in Norwalk, Ohio/USA, to find the link between the disease and norovirus [1]. The disease has become one of the greatest health problems of all infectious diseases throughout history. Nearly 90 million people is affected by the virus and virus-related disease and it claims about 1.5 million life annually around the globe and most of them are children [2-7]. Scientific efforts have so far failed to find an effective drug or an approved vaccine [8, 9].
Noroviruses carry the positive-strand RNA as genetic material and they are members of *Caliciviridae* family [10, 11]. There are mainly seven genogroups of noroviruses (GI-GVII) of which the GI, GII and GIV are the ones causing infection in the human [12, 13]. Since the virus develops mutations rapidly during its life cycle, these major groups are differentiated from each other and various genetic genotypes emerge such as GI.7, GII.4, GII.13, GII.17 etc. Although the involvement of factors for virus cell penetration have not fully been elucidated, studies so far have shown that the interaction between VP1 and HGBA on the host cell surface is required for infection [14-16]. This opens an attractive and hot research area that mainly focuses on finding and developing therapeutics which will block the interaction between VP1 and HBGA antigens for treating the disease [17-20]. With this scientific aim; several crystal structures of VP1-HGBA complex have been solved. Although these structures offer valuable information regarding the atomistic details of the interaction between the two entities, they lack of providing any insights about the dynamic changes upon ligand binding [16, 21-23].

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In our previous study, we found that the interaction between GI.1 VP1 and an H-type 1 HBGA antigen did not cause significant change in the binding region but showed a drastic change in a distal loop lying at 395-400 region [24].

Here, we computationally assessed the interaction between sugar part of A-type HGBA antigen and VP1 capsid protein utilizing molecular dynamics simulations. We also assessed whether the same type of dynamic changes upon A-type HBGA binding is observed as with our earlier findings.

2. MATERIALS AND COMPUTATIONAL METHODS

2.1. Protein Preparation

We used the Protein Data Bank (PDB id=2ZL7) as starting structures for halo-VP1 (complexed with A-type HBGA) [22]. The protein was prepared in Maestro [25] by assigning bonds, adding H atoms, fixing overlapping atoms and maximizing the number of H-bonds. The unprotonated side chains of Asp and Glu and protonated side chains of Arg and Lys residues were predicted by Propka 3.1 [26] at pH=7. We used the amber99sb-ildn [27] force field for the VP1 protein and GAFF force field for the ligand. The sugar moiety was first optimized using G09 software at B3LYP/6-311++G(d,p) level by fixing the heavy atoms at the x-ray coordinates. Then, the RESP charges and GAFF parameters were generated using antechamber software [28, 29].

2.2. Simulation Protocol

The simulations were performed using Gromacs 5.1 software package [30]. The dodecahedron box with a ~1500 nm^3 volume and the TIP3P model were used for solvation in simulations of both apo- and halo-form of VP1-HBGA complex systems [31]. Each system has ~145,000 atoms and neutralized by addition of 0.15 M NaCl. LINCS algorithm was used to restrain bonds with hydrogen atoms to their equilibrium length.

We minimized the system gently in 13 steps as with our earlier studies [32, 33]. Each step completed in 5000 cycles of two integrators, Steepest Descent and Conjugate Gradient. In the first step, only hydrogen atoms were kept free and relaxed while all heavy atoms including water frozen at a force of 4000 $kJ.mol^{-1}$. nm^{-2}. Then, the water molecules were relaxed. Next, the force on the side chains were slowly released by reducing the force constants in the order of 4000, 2000, 1000, 500, 200, 50 and 0 $kJ.mol^{-1}.nm^{-2}$. The minimization process was ended by releasing the backbone atoms gradually.

Each system was equilibrated within six steps following the minimization. The first step is a 5 ns of canonical ensemble. The system was heated to 310 K (with time constant of 0.1) with a simulated annealing manner in the first step. The temperature value was reached in the first 500 ps by linear heating and kept unchanged for the next 4.5 ns. For the protein-ligand complex and surroundings the V-rescale thermostat was used as the temperature-coupling group separately. Following the heating the system was equilibrated to the 1 atm pressure in a stepwise isobaric-isothermal ensemble. During this stage, the heavy atoms smoothly and progressively released. Five repetitive MD simulations with different initial velocities were run for 1 ns at the constant pressure using Langevin Dynamics.

3. RESULTS AND DISCUSSION

We have monitored the dynamic changes upon HGBA H-type 1 antigen in our previous study and observed a drastic movement in a distant loop [24].

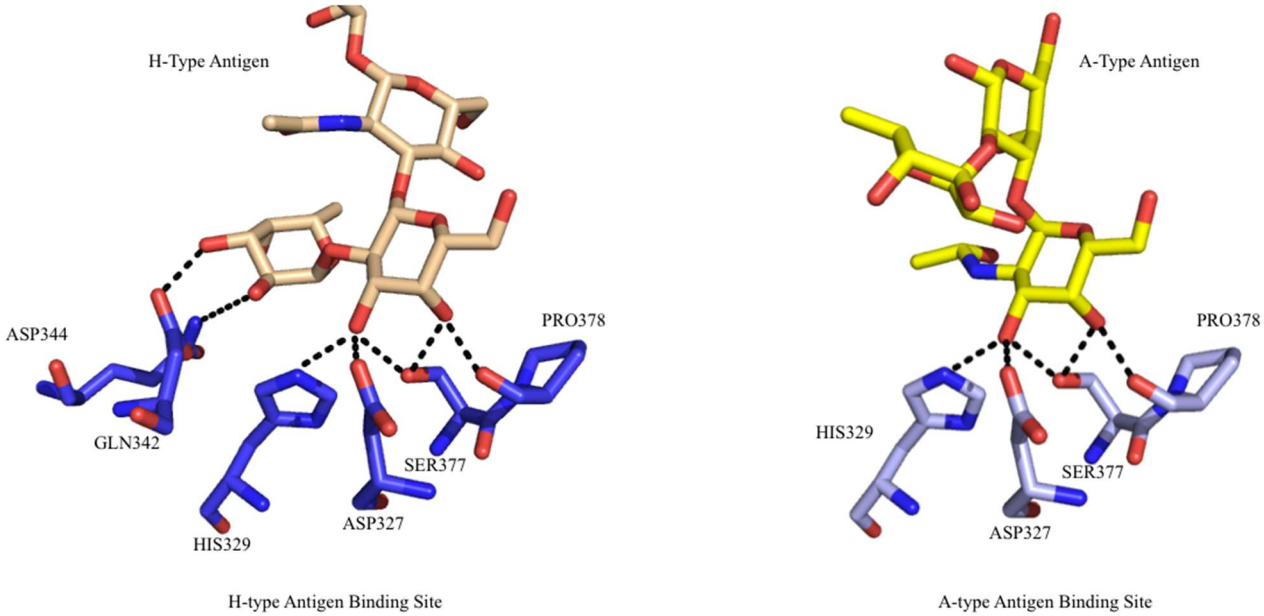

Figure 1 The sugar binding site of VP1 protein. Both A-type and H-type are binding at the same site.

In order to test whether this movement will be observed upon binding to other HBGAs, we simulated the dynamic changes of the complex formed by an A-type antigen and compared with apo form. Here on, all data regarding the apo form of VP1 is adopted from our previous study [24].

The main difference between A-Type and H-type HGBA antigens is that the FUC moiety in A-type antigen has missing interactions with VP1 capsid protein via Asp 344 and Gln 342 residues (Figure 1**).** Thus, it is expected to have a lower affinity in the A-type HBGA binding, which might influence the dynamic behavior.

The overall dynamic behavior of macromolecules can be followed by analyzing the RMSD values over time course of MD simulation. Therefore, we calculated the RMSD of GI.1 VP1 in complex with A type HGBA antigen (PDB id :2ZL7) and compared with apo GI.1 VP1 (PDB id:2ZL5). The ligand binding cause lower RMSD value in VP1 than apo form **(**Figure 2**).**

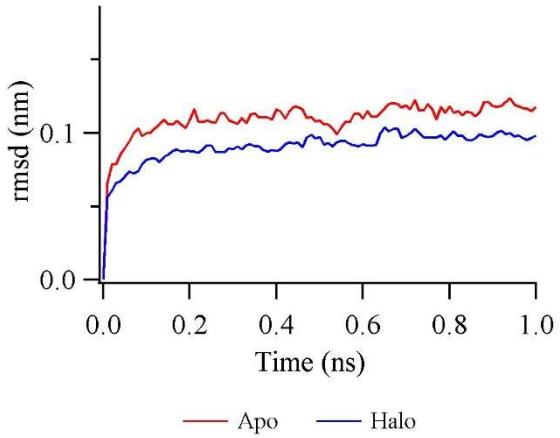

Figure 2 Rmsd values of both apo- and halo- forms of Cα atoms in GI.1 VP1 protein. Both the apo- and halo-forms follow similar trend.

The distances among residues that are near the sugar binding site in the crystal structure and MD simulation were computed for apo and halo form to follow any changes in this region (Table 1). These residues are in a direct contact with the sugar antigen within a distance maximum of 3.50 Å. We followed the deviation in the distances between interacting atoms of His329 and Trp375; Asp327 and Ser380; Trp375 and His329; Asp327 and Ser377; Trp375 and Gln342; Asp327 and Ser377 pairs, respectively, over the course of MD simulation upon sugar binding (Figure 3).

Table 1 Selected distances among the atom pairs of residues in the binding region. All values are in Å. The notation in distance is as follows: the first 3 digits show the residue number, rest shows the atom name in that residue (e.g., 375NE1 refers to Nε1 atom of 375th residue)

	X-ray crystal data		**MD simulation average**	
PDB id	2ZL5[a]	2ZL7[a]	2ZL5[b]	2ZL7[c]
	Apo-	Halo-	Apo-	Halo-
329O- 375N	3.29	3.26	3.48	3.42
327OD2-380OG	2.63	2.60	3.33	3.32
375O-329N	2.90	2.90	2.94	2.95
327O-377N	3.10	3.14	2.93	2.93
375NE1-342OE1	2.96	3.03	6.20	6.23
327N-377O	2.80	2.85	2.98	3.00
[a]: Ref. [22]; [b]:Ref. [24]; [c]: This study				

The distances between these residues are mostly conserved and show slight deviation throughout the simulation (Figure 3). The only notable change upon sugar binding take place in the distance between Asp327 and Ser380 residues. The distance between Asp327_OD2 and Ser380_OG is fluctuating in 1 Å oscillation range but this fluctuation is getting stabilized upon sugar binding (Figure 3b). This is an indication of insignificant dynamic changes due to ligand binding.

We have also analyzed the entire structures to figure out which part of protein gains or lose the flexibility upon ligand binding by analyzing the mean root square fluctuations (RMSF) of every single residue. The residues around the sugar binding site do not show remarkable fluctuations while some residues in a distant loop fluctuate drastically. (Figure 4). These fluctuations are consistent in all repetitive MD simulations for halo form while are not observed for apo protein. This rules out the possibility of a random loop movement.

We have investigated and confirmed this loop movement further by evaluating the principle component analysis (also called covariance analysis). The first eigenvalue from covariance analysis corresponds to this motion and the value in halo-form is very different from that of apo-form (Figure 5a). The rest of the eigenvalues follow similar trend in both proteins. This collective motion is more apparent in the rmsf of eigenvector 1. Comparing the halo-form RMSF with that of apo-form, the dominant factor contributing the 1st eigenvector is the drastic peak lying at 396-402 region. In addition, the residues lying at 303-316, 330-344 and 487-492 contribute to this eigenvector. However, fluctuations of these three regions were already observed in apo-form and thus are not sensitive to ligand binding. In addition, the ones at 294-300 and 303-316 region loses flexibilty upon ligand binding.

The virus genome is encapsulated by virus capsid which is formed by 180'mers (60 x trimer) of VPs. This capsid protects the genome from hostile environment. Once the capsid enters the cell, the capsid must be disassembled for the release of genome. The free genetic material is then translated to critical virus proteins by host cell machinery. Therefore, knowing the detail of capsid disassembly, which is known as uncoating mechanism, is another scientific interest in the field.

We assessed the location of the loop that become more flexible upon ligand binding. We have interestingly found that the loop resides between connection points of monomer VP building blocks that form the virus capsid which protects the virus genome against hostile environment **(Figure 7)**. The mobility of this loop may cause an instability in capsid particle which may be an evidence for uncoating mechanism of the virus.

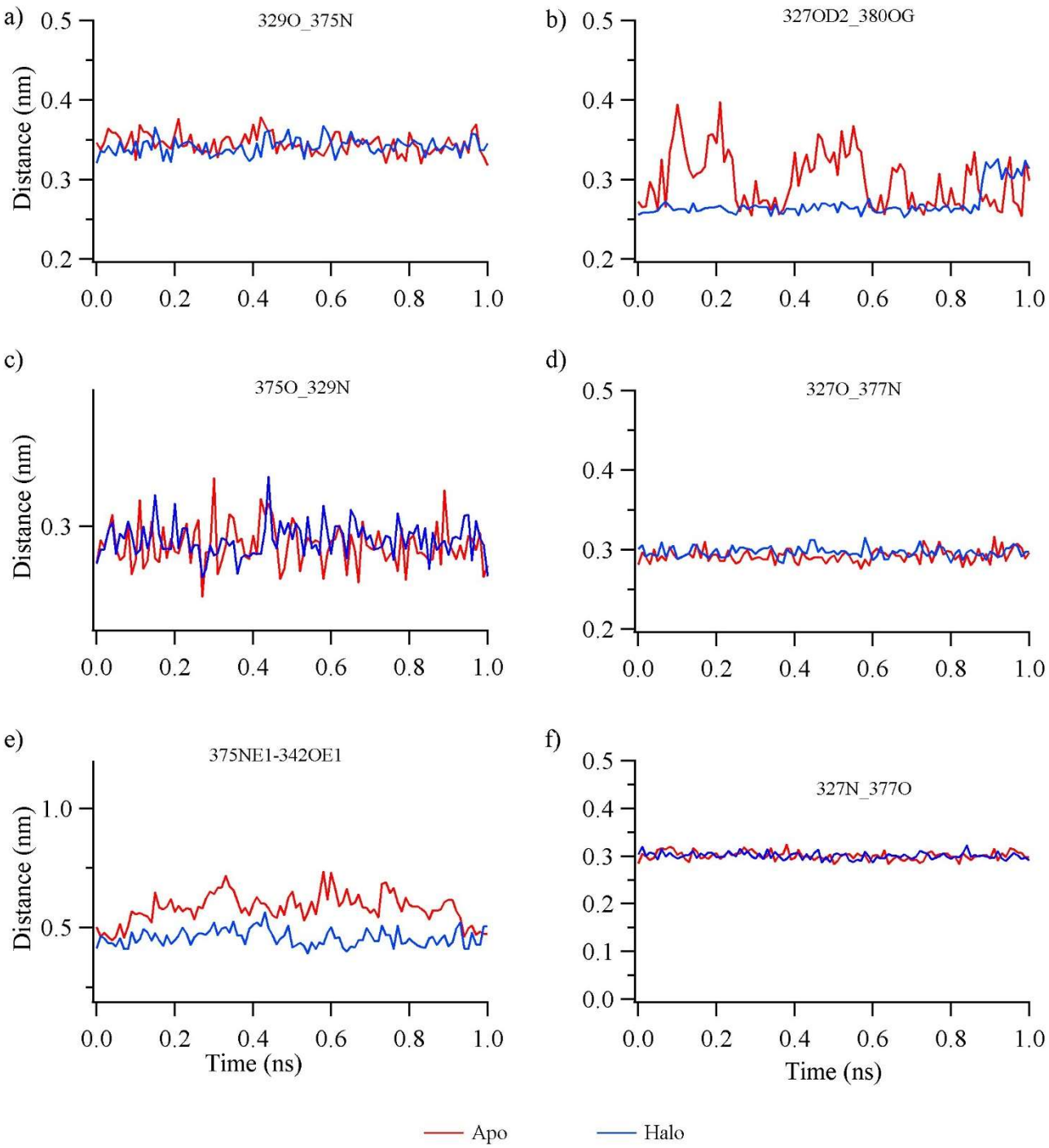

Figure 3 Selected distances among residues in the sugar binding site for halo-form (blue) and apo-form (red) proteins.

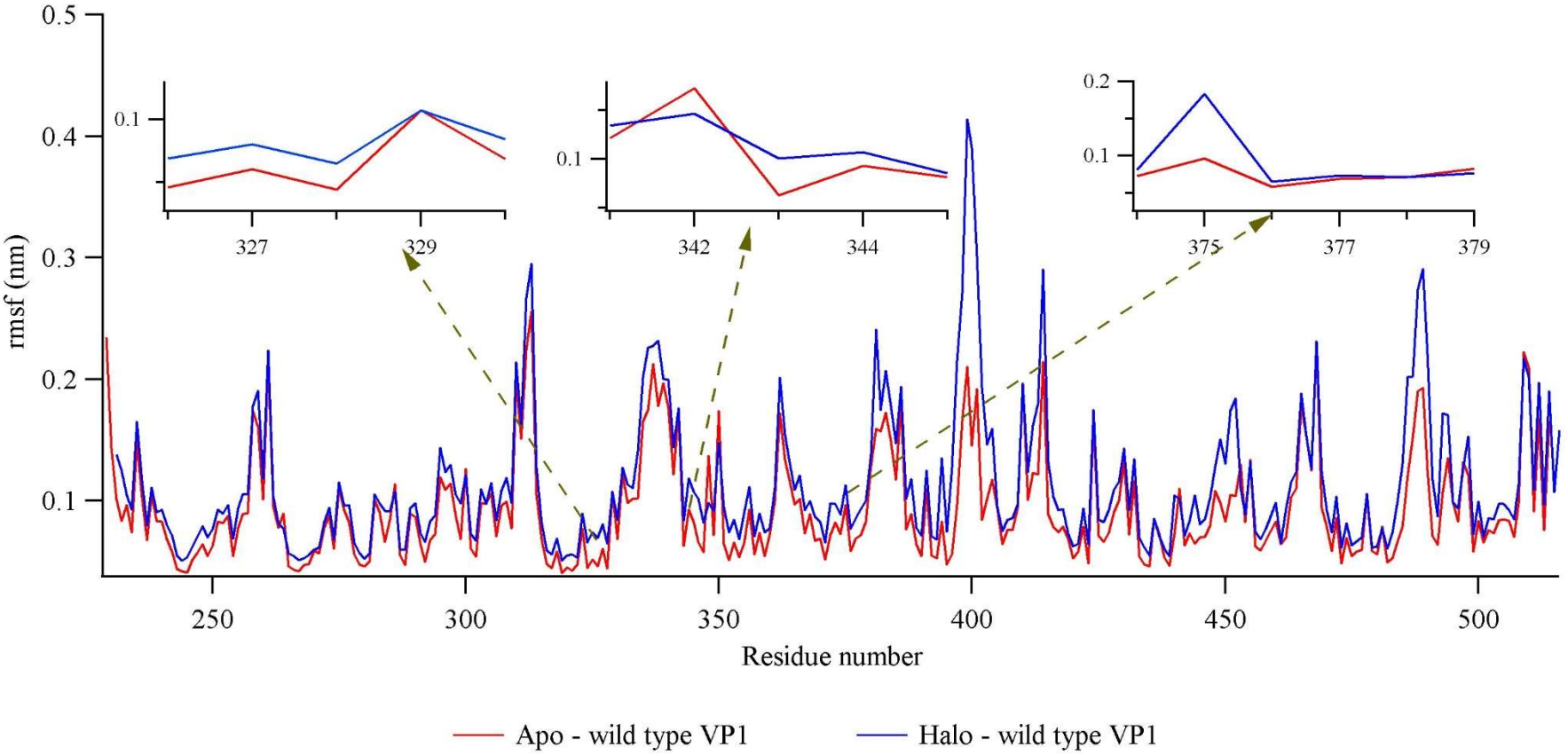

Figure 4 The rmsf of halo-form (blue) and apo-form (red) VP1 residues. The insets are the zoomed of the residues in the sugar binding site. Data do not show any significant rmsf difference at the binding site. The dashed arrows are not part of the data and only meant to show the location of zoomed insets (sugar binding site).

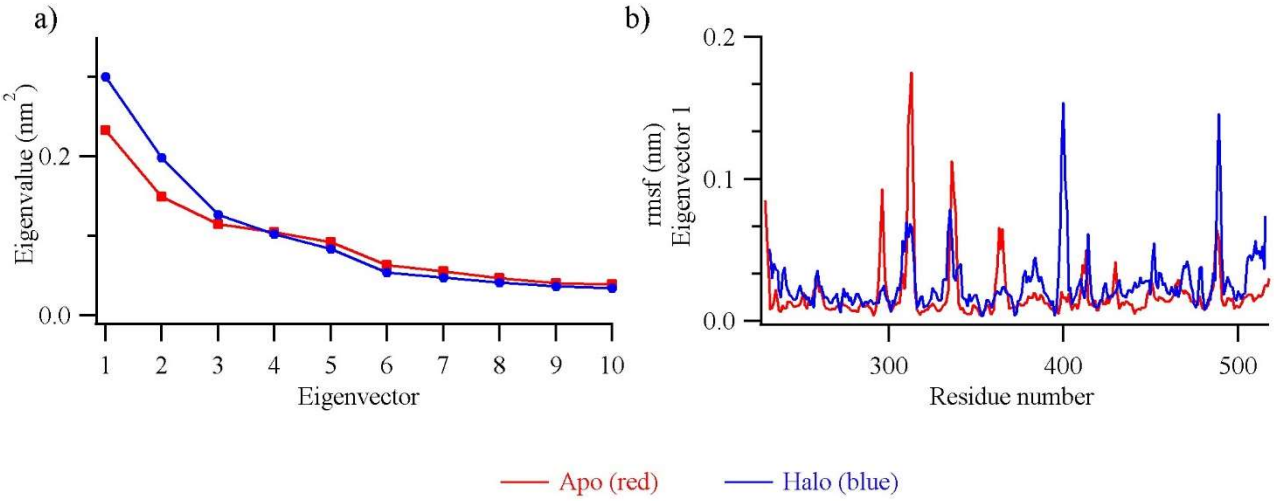

Figure 5 Covariance analysis of apo-form (red) and halo-form (blue) GI.1 VP1 proteins. a) The first 10 eigenvalues showing two proteins differ in only eigenvector 1 and 2. b) rmsf of residues involved in the most dominant collective motion (eigenvector 1)

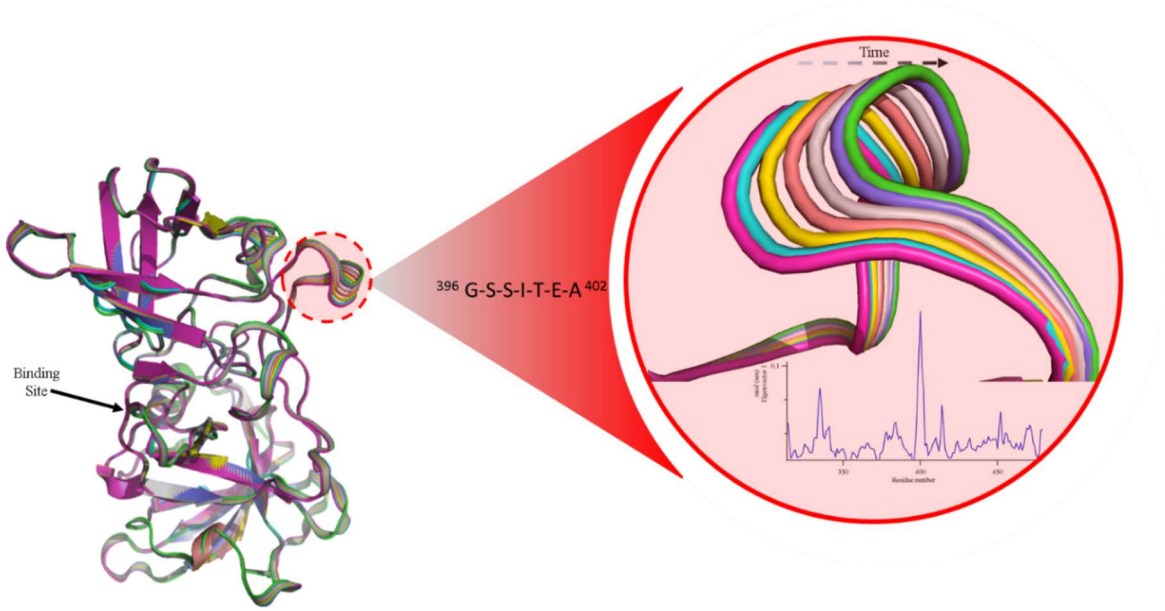

Figure 6 Allosteric loop fluctuation upon ligand binding

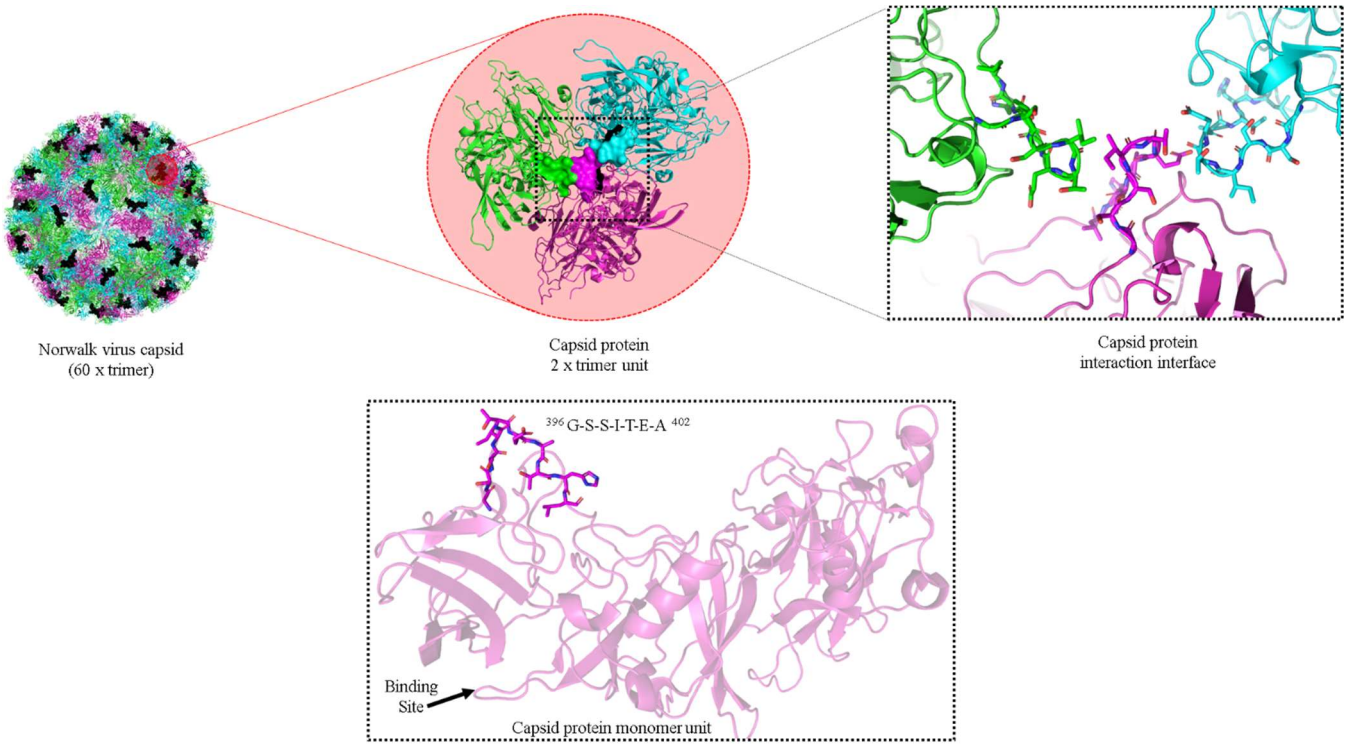

Figure 7 Norwalk virus capsid protein assembling organization from building blocks (PDB id=1IHM). The 396-402 loop stays in the building block interface and make critical interactions for capsid formation. This loop gains flexibility upon ligand binding while it is rigid in apo-form.

CONCLUSION

We have found that the interaction does not cause significant changes in the sugar binding site but stimulates a notable fluctuation in a distant loop. Interestingly this loop mobility also has been observed upon H-type antigen binding which is reported in our previous study. The loop stands in the interface of two VP1 protein that form a capsid for virus. Therefore, we assume that this mobility may help in virus un-coating during infection by destabilizing the capsid packing.

3.1. Appendix a: supplementary data

3.2. Acknowledgements

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