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An in-silico approach to identify avian IgY as potential inhibitor of HIV envelope glycoprotein gp120

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Abstract - *Human Immunodeficiency virus (HIV) is an endemic virus which attacks and degrades the human immune system, leading to illness. To infect the host cell HIV envelope glycoprotein gp120 binds to the cellular receptor CD4+ and co-receptor CCR5 or CXCR4. This results in fusion of the host cell membrane and viral leading to initiating infection. Blocking this interaction is a possible strategy to treat and prevent infection. For this work, Avian IgY (PDB ID: 2W59) is taken as antibody and HIV gp120 (PDB ID: 5F4I) as antigen. To study antibody-antigen interaction, modelling of antibody (Avian IgY) and antigen (HIV gp120) has been done and docking is performed. Modelled structures were verified by ERRAT and Verify_3D.*

Key Words: **avian IgY, gp120, QMEAN4 score, ClusPro 2.0, ERRAT2, Antibody-Antigen docking, Verify_3D etc.**

1. INTRODUCTION

Every year approximately 2-3 million people are infected with HIV [1]. HIV is a retrovirus that attacks the body's natural defence mechanism and weakens the immune system. The virus destroys the white blood cell called a T-helper cell and makes multiple copies of itself inside the t-cell. Due to this person's immune system gradually breaks down, leading to AIDS. This is the condition in which a person can be infected with vulnerable to other infected diseases. HIV transmission can happen mainly due to sexual intercourse, prenatal transfusion, and blood transfusion. In order to replicate HIV must enter a cell. For infection, a series of protein-protein interaction is performed. Once the virus gets inside the cell, the replication progress and this result in the production of progeny virus. That's, why blocking the virus from entering the cell is one of the ways to stop further replication and production of progeny virus [1]. Specific entry inhibitors are sometimes used alone or with other drugs to stop infection to the new cell in HIV infected patient. The envelope glycoprotein (env) mediates the HIV entry by attacking to specific cell surface receptors CD4+ and co-receptors CCR5 or CXCR4, followed by fusion. The chemoreceptors CCR5 and CXCR4 classified the HIV strain in R5 and X4. The env is produced from gp160, which is cleaved by furin-like protease. This cleavage produces a trans membrane moiety gp41 and an outer subunit gp120 [1]. Gp120 contains five variable loops (V_1-V_5), formed by a disulfide bond at it based, and five relatively conserved domain (C_1-C_5) [2]. The variable lops have the critical role in co-receptor binding and immune eluding, and are usually lie at the surface. The variable regions V_1, V_2 and V_3 are rearranged due to the attachment of gp120 to cellular receptors and co-receptors [2]. Designing the antigens or immunogens for the virus is a great interest among the scientist. One of the ways is the production of the antibody with the help of avian and their eggs. One of them is the production of IgY antibody which can be extracted the avian blood and also be extracted from egg yolk in large quantities [3]. The function of IgY is similar to the mammalian IgG in terms function but is different in structure, and physiochemical and immunological capability. The extracted IgY from avian blood and its egg has the potential to be an anti-HIV agent [4-6]. Binding interaction between antigen and protein can be studied by docking to predict the complex structure [7]. Docking is one of the frequently used methods to predict the binding orientation of antibody and small molecule to their protein targets such as antigens [8]. To predict a chemical characteristics and the behaviour of molecule, molecular modelling is used to construct a molecule by the use of the computer.

2. METHODOLOGY

Sequence retrieval and analysis: The protein sequence of Avian IgY-Fc (PDB ID: 2W59) [9] and gp120 (PDB ID: 5F4L) [10] were retrieved from the RSCB Protein Data Bank (https://www.rcsb.org/) in FASTA format.

Sequence analysis and homology modeling of Avian IgY and gp120: The three-dimensional structure of Avian IgY and gp120 were modeled with the swiss modeler web server (http://swissmodel.expasy.org/). Swiss modeler is a fully automated protein structure homology modeling server and it requires the query sequence in FASTA format. Template search, template selection, model build and model estimation, all are done by the swiss modeler automated server.

Evaluation and validation of models: The final selected model of avian antibody IgY and antigen gp120, generated from swiss modeler were selected on the basis of QMEAN4 score. Validation (ERRAT and Verify3D) of the proposed models were done in SAVES server (https://services.mbi.ucla.edu/SAVES/), and feasibility estimation (Procheck statistics) was carried out in PDBsum web server.

Antibody-Antigen docking: The antibody-antigen docking was carried out by ClusPro 2.0 (https: //cluspro.bu.edu/login.php) in antibody mode [11-15]. This server is a fully automated web-based molecular docking program.

3. RESULT AND DISCUSSION

Modeling and assessment of modeled protein structures: Swiss modeler server searches the template with BLAST (Altschul, et al., 1997) and HHBlits (Remmert, et al., 2011) against the swiss-model template library (SMTL). The template's quality has been predicted for each identical template from the feature of the target template alignment and the templates with the highest quality have been selected for modeling. Models are built by the server using ProMod3, based on the target template alignment, and in case of loop modeling an alternative model is built with PROMOD-II (Gus, et, al., 1997). The global and per-residue model quality estimation is done using QMEAN scoring function (Benkert, et al., 2011). Modeled proteins structures antigen gp120 and antibody Avian IgY have the QMEAN4 scores -0.34 and 0.58 subsequently.

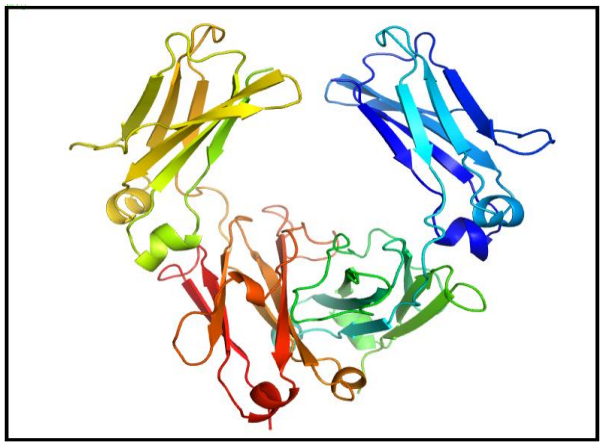


Fig-1: Modelled structure of avian antibody IgY.

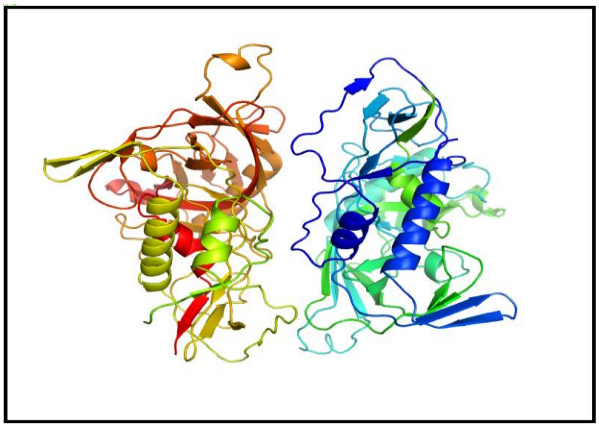


Fig-2: Modelled structure of HIV gp120.

SAVES server tool such as Verify_3D determines the atomic model compatibility, by assessing a structural class based on its location and environment (alpha. beta, loop, polar, nonpolar etc.) and comparing the result to good structures [16]. According to Verify_3D criterion, the modelled structure of HIV gp120 was 88.25 % acceptable and the modelled structure of avian antibody IgY was 90.37% acceptable (Bowie, et al., 1991) (Luethy, et al., 1992). The ERRAT2 program gave the overall quality factor 93.68 for HIV gp120 and 82.58 for antibody IgY, these indicate that the models are acceptable. The ProCheck indicated that, according to psi and phi torsion angles. 94.3% residues were in the most favoured region and 5.7% residues were in the additional allowed region for antibody IgY, and 90.6% residues were in most favoured region, 8.6% in additional allowed region, and 0.5% in generously allowed region. Also, G-factor shows that the models' properties were usual [17-20].

Procheck statistics of modeled avian IgY:

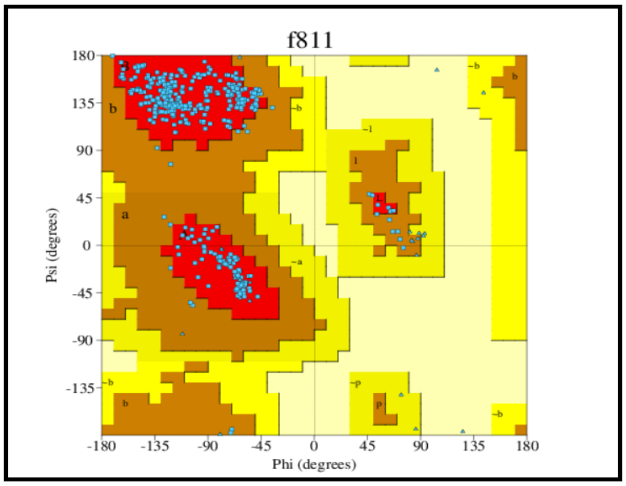
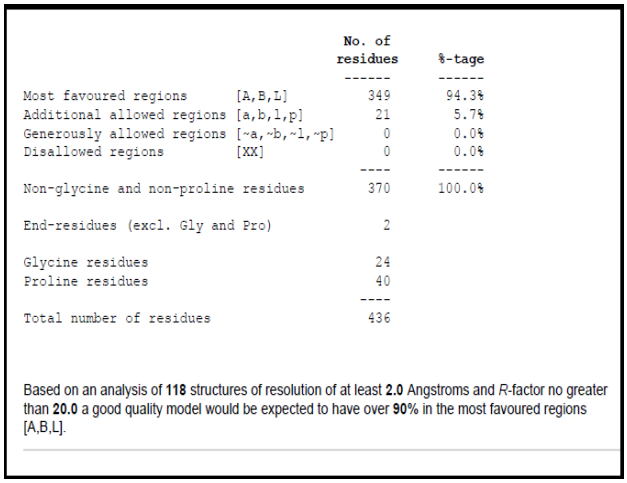


Fig-3: Ramachandran Plot of avian antibody IgY.



		No. of residues	%-tage
Most favoured regions	[A,B,L]	349	94.3%
Additional allowed regions	[a,b,l,p]	21	5.7%
Generously allowed regions	[~a,~b,~l,~p]	0	0.0%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		370	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		24	
Proline residues		40	
Total number of residues		436	

Based on an analysis of **118** structures of resolution of at least **2.0** Angstroms and *R*-factor no greater than **20.0** a good quality model would be expected to have over **90%** in the most favoured regions [A,B,L].

Fig-4: Ramachandran plot statistics

Parameter	Score	Average Score
Dihedral angles:-		
Phi-psi distribution	-0.20	
Chi1-chi2 distribution	0.23	
Chi1 only	-0.01	
Chi3 & chi4	0.71	
Omega	-0.73*	
		-0.19
Main-chain covalent forces:-		
Main-chain bond lengths	0.35	
Main-chain bond angles	-0.07	
		0.11
OVERALL AVERAGE		-0.06

G-factors provide a measure of how **unusual**, or out-of-the-ordinary, a property is.

Values below -0.5* - unusual
Values below **-1.0**** - highly unusual

Important note: The main-chain bond-lengths and bond angles are compared with the Engh & Huber (1991) ideal values derived from small-molecule data. Therefore, structures refined using different restraints may show apparently large deviations from normality.

Fig-5: G-factors

Parameter	Score	Average Score
Dihedral angles:-		
Phi-psi distribution	-0.33	
Chi1-chi2 distribution	-0.02	
Chi1 only	-0.03	
Chi3 & chi4	0.50	
Omega	-0.62*	
		-0.25
Main-chain covalent forces:-		
Main-chain bond lengths	0.22	
Main-chain bond angles	-0.22	
		-0.04
OVERALL AVERAGE		-0.16

G-factors provide a measure of how **unusual**, or out-of-the-ordinary, a property is.

Values below -0.5* - unusual
Values below **-1.0**** - highly unusual

Important note: The main-chain bond-lengths and bond angles are compared with the Engh & Huber (1991) ideal values derived from small-molecule data. Therefore, structures refined using different restraints may show apparently large deviations from normality.

Fig-8: G-factors.

Procheck statistics of modeled HIV gp120:

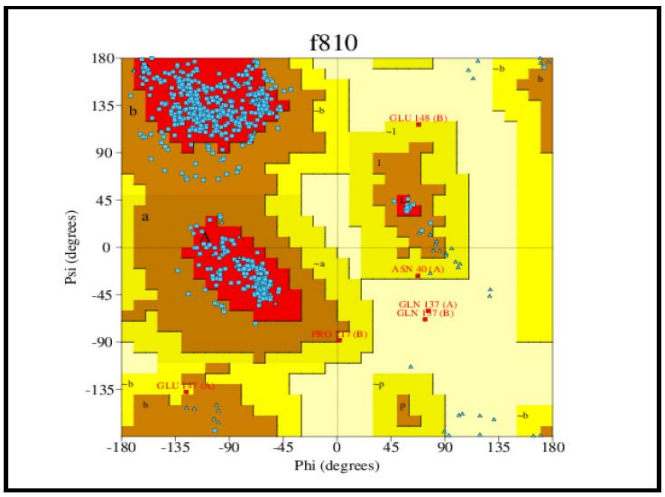


Fig-6: Ramachandran Plot of HIV gp120.

		No. of residues	%-tage
Most favoured regions	[A,B,L]	550	90.6%
Additional allowed regions	[a,b,l,p]	52	8.6%
Generously allowed regions	[~a,~b,~l,~p]	3	0.5%
Disallowed regions	[XX]	2	0.3%*
Non-glycine and non-proline residues		607	100.0%
End-residues (excl. Gly and Pro)		6	
Glycine residues		56	
Proline residues		32	
Total number of residues		701	

Based on an analysis of **118** structures of resolution of at least **2.0** Angstroms and *R*-factor no greater than **20.0** a good quality model would be expected to have over **90%** in the most favoured regions [A,B,L].

Fig-7: Ramachandran plot statistics.

Antibody-Antigen docking: ClusPro 2.0 web server uses a supercomputer for protein-protein docking at Boston University. This server which is developed by the groups of Sandor Vajda and Dima Kozakov was the best in the server category in the latest rounds of CAPRI experiment [21]. The best result is selected based on the lowest energy calculated ($E=0.50E_{rep}+-0.20E_{att}+600E_{elec}+0.25E_{DARS}$ [14]); which was -355 Kcal/mol. This energy lowering in the antibody–antigen complex was mainly due to stabilizing by hydrophobic interactions and hydrogen bonding. Also due to the Vander Walls' interactions and groups o charged and polar residues lead to the stability of the complex. Multiple bonding between the antibody and antigen ensures that antigen will be tightly bound to the antibody. Bonds were located in the epitope of antigen and CDR region of the antibody. Though, two Gly 182 and 183 are not in CDR region. Among the amino acid of antigen (HIV gp120) that interact with paratope, Asn 248, Lys 278, Asn 188, and Ser 185 were in epitope region, but amino acids Arg 258, Asn263, Glu 224, Asp 320, Asn 254, Thr 265 and Gly 266were not in this region. Bonds between amino acids, ser 28 - Arg 258, Asp 30 - Ag 258, Asp 30- Asn 263, Glu 24- Asn 263 , Arh 59- Glu 324, Arh 59 - Asp 320, Lys 32 - Asn 254, ASP 61 - Asn 254, Arg 34 - Thr 265, Arg 34 - Gly 266, Leu 65 -Asn 28, Tyr 73 -Lys 278, Glu 67 - Lys 278, Glu 67 - Asn 188, Phe 69 - Ser 185, Gly 183 - Pro 21, and Gly 182 - Asn 259 were seen.

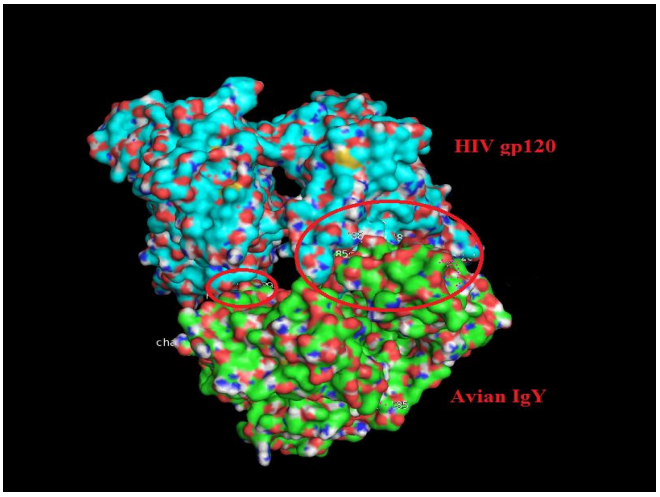


Fig-9: Surface interaction between HIV gp120 and Avian IgY.

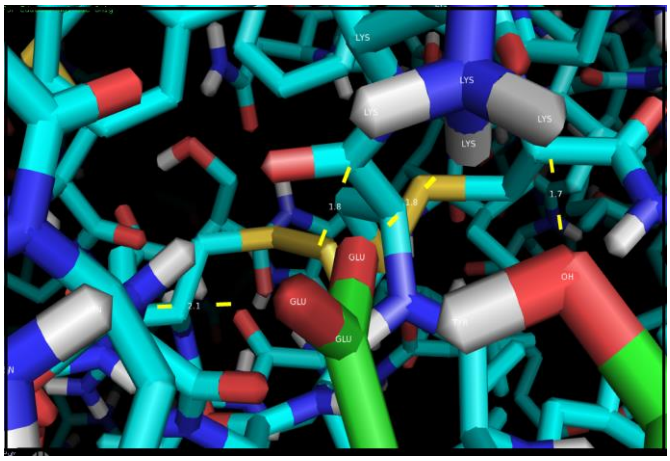


Fig-10: Interaction between HIV gp120 and Avian IgY with the hydrogen bond.

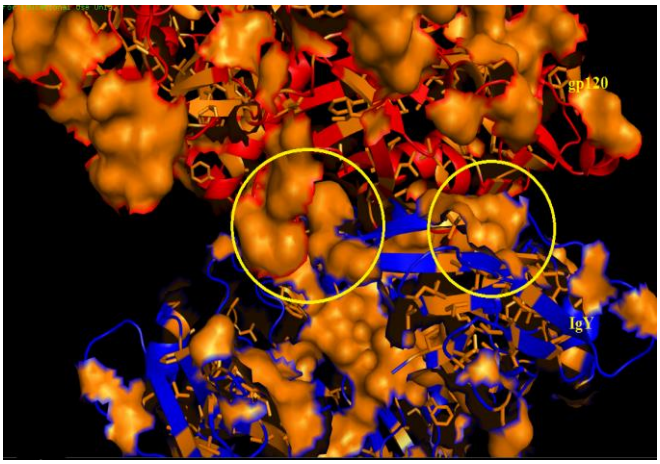


Fig-11: Hydrophobic Interaction (in brown color) between HIV gp120 and Avian IgY.

4. CONCLUSIONS

The Avian antibody IgY was well modeled with respect to the assessments made on its structure. Also, protein-protein docking result revealed the high affinity of antibody IgY and gp120 antigen. Hydrogen bonds, Vander Wall's interaction, and hydrophobic interactions were found to have a great role in the interaction between antibody and antigen. It can be concluded that the avian antibody IgY can be used to inhibit HIV gp120 and treat HIV patients.

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