

Identification of 3D Structure of Gag Polyprotein HIV Type 1 Group M Subtype B isolate HXB2 a Promising Drug Target by Threading Assembly Refinement

Undety Benjamin Jason¹ and Daniel Alex Anand²

¹Department of Bioinformatics, Sathyabama University, Chennai, India.

²Department of Biomedical Engineering, Sathyabama University, Chennai, India.

doi: <http://dx.doi.org/10.13005/bbra/1343>

(Received: 08 June 2014; accepted: 08 July 2014)

The discovery of potential drug to arrest the replication of HIV-1 is an ever increasing task. There are drugs mostly targeting the three enzymes reverse transcriptase, integrase and protease. Gag gene of HIV genome GAG is found to be more conserve. Functional conservation of HIV-1 Gag implicates rational drug design [1]. HIV-1 replication can be successfully blocked by targeting gag gene polyprotein, offering a promising strategy for new drug classes that complement current HIV-1 treatment options. Gag's role being responsible forming encapsulation of the virus if disturbed the whole Virus is gone. So the challenge is now to determine the structure of Gag poly-protein as it is undetermined still. There is no crystal structure available in the PDB database. The gag polyprotein of Human immunodeficiency virus type 1 group M subtype B (isolate HXB2) (HIV-1) is downloaded for three dimensional structure determinations. The unavailability of a close template is an added challenge for alternative structure prediction. The non availability of PDB structure and similar template with higher number of residues caused to use threading assembly refinement. This work further carries in finding active site, lead screening and docking studies of Gag poly protein.

Key words: Conservation, gag polyprotein, target, template.

HIV-1, which causes acquired immune deficiency syndrome (AIDS), is a retrovirus in genus Lentiviridae. HIV-1 is an enveloped virus which encodes two envelope (Env) glycoproteins - the surface (SU) glycoprotein gp120 and a transmembrane (TM) glycoprotein gp41, Gag has four major proteins, they are matrix (MA), capsid (CA), nucleocapsid (NC), and p6—and the pol-encoded enzymes protease (PR), reverse transcriptase (RT), and integrase (IN). HIV-1 also encodes two regulatory proteins, they are Tat and Rev and several accessory proteins, they are Vpu,

Vif, Nef, and Vpr. The genome is pseudodiploid which is composed of two single strands of RNA linked in dimer. The HIV-1 infection initiates with the attachment of gp120 to target cell plasma membrane.¹⁻⁴ The principal attachment of the receptor for HIV-1 and other pri-mate lentiviruses is CD4. Productive infection also requires the presence of a co-receptor, they are typically CXCR4 or CCR5. The binding of gp120 to CD4 and co-receptor initiates conformational changes in gp41, which in turn directs to fusion of the viral envelope and the target cell membrane and entry of the viral core into the host cell cytoplasm. Recent evidence suggests that HIV-1 entry can also occur in a low-pH endosomal compartment after receptor-mediated endocytosis.⁵ Upon entry of the virion into the cytosol, the Env glycoproteins and the lipid-associated MA protein dissociate from

* To whom all correspondence should be addressed.

the incoming particle at the membrane, and the poorly understood process of uncoating is initiated. The enzymes RT and IN, together with the NC protein, remain in close association with the viral RNA as it is converted to double-stranded DNA by RT-catalyzed reverse transcription.⁶ NC acts as a nucleic acid chaperone at several steps during reverse transcription to facilitate the conversion of RNA to DNA.⁷ Vpr is also a component of the reverse transcription complex (RTC). The extent to which CA remains associated with the incoming RTC has been a topic of debate. However, reverse transcription and uncoating appear to be temporally linked,⁸ and it is clear that some host restriction factors that block early postentry steps in the viral replication cycle target CA.^{9,10} The newly reverse transcribed viral DNA is translocated to the nucleus in a structure known as the preintegration complex (PIC). The nuclear import process remains incompletely understood; however, a role for CA in this process^{11,12} implies that some CA protein may remain associated with the viral nucleoprotein complex as it trafics to the nuclear pore. Once inside the nucleus, the double-stranded viral DNA integrates into the target cell genome through the action of the IN enzyme.¹³ The integrated viral DNA serves as the template for transcription from the viral promoter in the 5' long terminal repeat (LTR) to generate the spliced viral mRNAs and full-length genomic RNAs; these are transported out of the nucleus via the action of the Rev protein.³ The Gag proteins are translated from full-length message as a polyprotein precursor containing MA, CA, NC, and p6 domains as well as two spacer peptides, SP1 and SP2.¹⁵ During translation of the Gag precursor, known as Pr55^{Gag}, an occasional 1 ribosomal frameshift leads to the production of a GagPol precursor protein (Pr160^{GagPol}), the abundance of which is approximately 5% that of Pr55^{Gag}. The Gag and GagPol precursor polyproteins are transported to the plasma membrane, where they assemble and incorporate the viral Env glycoproteins. The membrane targeting of Gag and GagPol is regulated by the MA domain, which also plays an important role in the incorporation of the viral Env glycoproteins. Assembly takes place in cholesterol-rich membrane microdomains (lipid rafts) through direct interactions between MA and the phospholipid phosphatidylinositol-4,5-

bisphosphate [PI(4,5)P2].¹⁷ Interactions within the CA domain of Gag initiate the Gag assembly process.

The Gag gene of HIV-1 expressed MA (p17), CA (p24), SP1 (p2), NC (p7), SP2 (p1) and P6. HIV p6 is a 6 kDa polypeptide at the N-terminus of the Gag polyprotein. It recruits cellular proteins Tsg101 (a component of ESCRT-1) and Alix to initiate virus particle budding from the plasma membrane. p6 has no known function in the mature virus. P6 protein was taken to study its variance in sequence and structural levels. Gag proteins play a vital role in virus assembly, release, maturation, function in the establishment of a productive HIV-1 and which are also viral structural proteins. In spite of their vital role throughout the replication cycle, there are currently no approved antiretroviral therapies that target the Gag precursor protein or any of the mature Gag proteins. Recent progress in understanding the structural and cell biology of HIV-1 Gag function has revealed a number of potential Gag-related targets for possible therapeutic intervention. In this study, we emphasize that our current understanding of HIV-1 Gag P6 protein suggests some approaches to be as a target for novel antiretroviral agents.

MATERIALS AND METHODS

Sequence of Gag Poly Protein: The gag polyprotein of Human immunodeficiency virus type 1 group M subtype B (isolate HXB2) (HIV-1) is downloaded for three dimensional structure determinations. Its length is 500 amino acids. The Gag polyprotein sequence is retrieved from the Uniprot database, >sp|P04591|GAG_HV1H2 Gag polyprotein OS=Human immunodeficiency virus type 1 group M subtype B (isolate HXB2) GN=gag PE=1 SV=3

M G A R A S V L S G G E L D R W E K I R L R P G
G K K K Y K L K H I V W A S R E L E R F A V N
P G L L E T S E G C R Q I L G Q L Q P S L Q T G S E E L
R S L Y N T V A T L Y C V H Q R I E I K D T K E A L D K I
E E E Q N K S K K K A Q Q A A A D T G H S N Q V S Q N
Y P I V Q N I Q G Q M V H Q A I S P R T L N A W V K V V E E
K A F S P E V I P M F S A L S E G A T P Q D L N T M L N T V
G G H Q A A M Q M L K E T I N E E A A E W D R V H P
V H A G P I A P G Q M R E P R G S D I A G T T S T L Q
E Q I G W M T N N P P I P V G E I Y K R W I I L G L N K I V
R M Y S P T S I L D I R Q G P K E P F R D Y V D R F Y K

TLRAEQ ASQEVK NWMTETLLVQNANP
 DCKTILKALG P AATLEEMMT
 ACQGVGGPGHK ARVLAEA MSQVTSATI
 MMQRGNFRNQ RKIVKCFN CGKEGHT
 ARNCRAPRKKGCW KCGKEGHQMKD
 CTERQANFLGKIWPSYKGR PGNFLQSRP
 EPTAPPEESFRSGV ETTTPPQKQ EPIDKE L
 YPLTSLRSLFGNDPSSQ

A hierarchical protein structure modeling approach is used on the secondary-structure enhanced profile-profile threading alignment and the iterative implementation of the threading assembly refinement program³. Critical Assessment of Structure Prediction (CASP) experiment implemented. CASP (or Critical Assessment of Techniques for Protein Structure Prediction) is a community-wide experiment for testing the state-of-the-art of protein structure predictions which takes place every two years since 1994. The experiment (often referred as a competition) is strictly blind because the structures of testing proteins are unknown to the predictors.



Fig. 1. Predicted structure of Gag polyprotein

well as to refine the global topology of the cluster centroids. The decoys generated in the second simulations are then clustered and the lowest energy structures are selected. The final full-atomic models are obtained by REMO which builds the atomic details from the selected I-TASSER decoys through the optimization of the hydrogen-bonding network. The three dimensional structure is determined with the 89.8% of amino acids in favored regions by Rampage.

RESULTS AND DISCUSSION

The software first retrieves template proteins of similar folds (or super-secondary structures) from the PDB library by LOMETS, a locally installed meta-threading approach. Then the continuous fragments excised from the PDB templates are reassembled into full-length models by replica-exchange Monte Carlo simulations with the threading unaligned regions (mainly loops) built by ab initio modeling. In cases where no appropriate template is identified by LOMETS, I-TASSER will build the whole structures by ab initio modeling. The low free-energy states are identified by SPICKER through clustering the simulation decoys. In the third step, the fragment assembly simulation is performed again starting from the SPICKER cluster centroids, where the spatial restrains collected from both the LOMETS templates and the PDB structures by TM-align are used to guide the simulations. The purpose of the second iteration is to remove the steric clash as

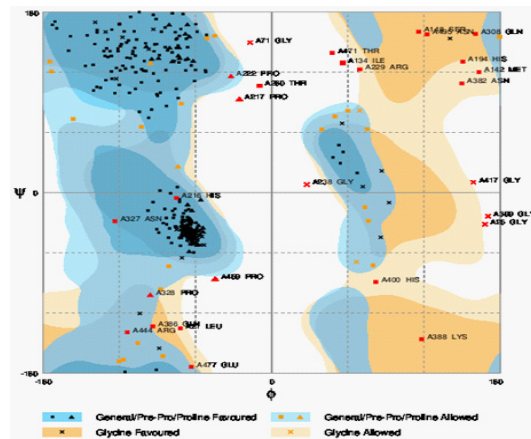


Fig. 2. Ramachandran diagram of the best model

The best predicted model evaluated by Rampage, University of Cambridge is shown in Fig2 which was found to have best score being validated out of the five models.

The drug resistance to Anti-retroviral therapy targeting mostly RT and IN has directed to study Gag poly protein. The Gag polyprotein was understood to be conserved and implicates rational drug design. As it forms the encapsulation of HIV, if blocked it allows core viral ingredients to fatal end. The non availability of PDB structure and similar

Number of residues in favoured region (~98.0% expected) : 446 (89.6%)
 Number of residues in allowed region (~2.0% expected) : 25 (5.0%)
 Number of residues in outlier region : 27 (5.4%)

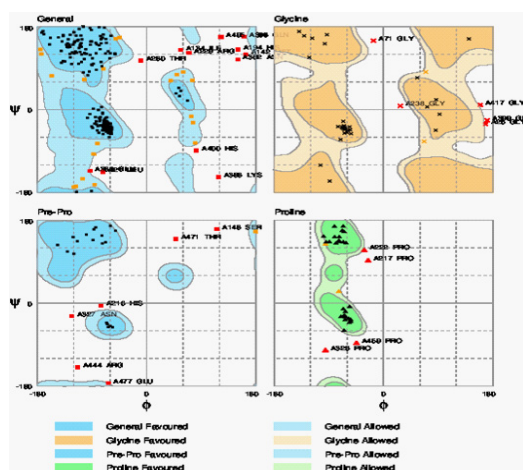


Fig. 3. Residues that fell into the unfavoured region

template with higher number of residues caused to use threading assembly refinement. This work further carries in finding active site, lead screening and docking studies of Gag poly protein.

REFERENCES

- Guangdi Li, Jens Verheyen, Soo-Yon Rhee, Arnout Voet, Anne-Mieke Vandamme, and Kristof heys: Functional conservation of HIV-1 Gag: implications, *Retrovirology*, 2013, 10:126.
- Wu ST, Zhang Y: LOMETS: A local meta-threading-server for protein structure prediction. *Zhang Y, Skolnick J: Automated structure prediction of weakly homologous proteins on a genomic scale.*
- Melikyan GB: Common principles and intermediates of viral protein-mediated fusion: The HIV-1 paradigm. *Retrovirology* 2008; **5**:111.
- Miyauchi K, Kim Y, Latinovic O, Morozov V, and Melikyan GB: HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes. *Cell* 2009; **137**(3):433–444.
- Telesnitsky A and Goff SP: Reverse transcriptase and the generation of retroviral DNA. In: *Retroviruses* (Coffin J, Varmus H, Hughes S, eds.). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1997; 121–160.
- Levin JG, Guo J, Rouzina I, and Musier-Forsyth K: Nucleic acid chaperone activity of HIV-1 nucleocapsid protein: Critical role in reverse transcription and molecular mechanism. *Prog Nucleic Acid Res Mol Biol* 2005; **80**:217–286.
- Hulme AE, Perez O, and Hope TJ: Complementary assays reveal a relationship between HIV-1 uncoating and reverse transcription. *Proc Natl Acad Sci USA* 2011; **108**(24): 9975–9980.
- Song B: TRIM5alpha. *Curr Top Microbiol Immunol* 2009; **339**: 47–66.
- Towers GJ: The control of viral infection by tripartite motif proteins and cyclophilin A. *Retrovirology* 2007; **4**:40.
- Dismuke DJ and Aiken C: Evidence for a functional link between uncoating of the human immunodeficiency virus type 1 core and nuclear import of the viral preintegration complex. *J Virol* 2006; **80**(8): 3712–3720.
- Yamashita M and Emerman M: Capsid is a dominant determinant of retrovirus infectivity in nondividing cells. *J Virol* 2004; **78**(11): 5670–5678.
- Brown PO: Integration. In: *Retroviruses* (Coffin J, Varmus H, Hughes S, eds.). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1997, pp. 161–205.
- J Gen Virol. 2006 Jul; **87**(Pt 7):2041-6. Effects of human immunodeficiency virus type 1 transframe protein p6* mutations on viral protease-mediated Gag processing. Chiu HC, Wang FD, Chen YM, Wang CT. Source, Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan.
- Structure and assembly of immature HIV J. A. G. Briggs, I. J. D. Richesa, B. Glassb, V. Bartonovab, G. Zanettia, c and H.-G. Kräusslichb, + Author Affiliations
- J Virol. 1995 Nov; **69**(11):6810-8. p6Gag is required for particle production from full-length human immunodeficiency virus type 1 molecular clones expressing protease. Huang M, Orenstein JM, Martin MA, Freed EO. Source, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892-0460, USA.
- Proline residues in human immunodeficiency virus type 1 p6(Gag) exert a cell type-dependent effect on viral replication and virion incorporation of Pol proteins. Dettenhofer M, Yu XF. Source, Department of Molecular Microbiology and Immunology, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205, USA.
- S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson and D.C. Richardson., Structure validation by C α geometry: phi, psi and C β deviation. *Proteins: Structure, Function & Genetics*. 2002; **50**: 437-450.

19. Yang Zhang. I-TASSER server for protein 3D structure prediction. *BMC Bioinformatics*, 2008; **9**: 40.
20. Amrisha Roy, Alper Kucukural, Yang Zhang. I-TASSER: a unified platform for automated protein structure and function prediction. *Nature Protocols*, 2010; **5**: 725-738.
21. Amrisha Roy, Jianyi Yang, Yang Zhang. COFACTOR: an accurate comparative algorithm for structure-based protein function annotation. *Nucleic Acids Research*, 2012; **40**: W471-W477.