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Structural Studies of Computationally Designed Inhibitors of HIV Gp41

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Abstract

Human immunodeficiency virus (HIV) infection is a major public health concern. New strategies that target viral entry hold promise for preventing infection or for enhancing the efficacy of existing anti-retroviral therapy. We have targeted the HIV protein gp41, required for initial membrane fusion and viral entry, for computational lead compound development and structural characterization. We have successfully purified an engineered version of this protein (gp41-5) that has an exposed patch that resembles the pre-fusion complex that is presumed to be an important intermediate in fusion. We crystallized this protein and collected a complete dataset, however the crystals suffer from an unusual order-disorder pathology. Current work is identifying improved crystallization conditions that will allow determination of the crystal structure of gp41-5 with computationally identified compounds that inhibit viral entry.

Introduction

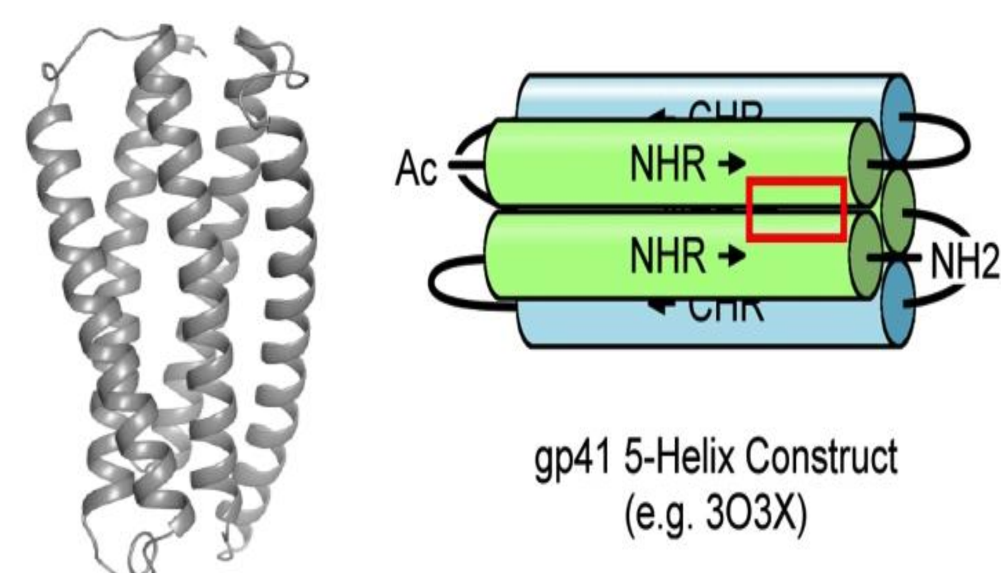
The human immunodeficiency virus (HIV) is a retrovirus that causes acquired immune deficiency syndrome (AIDS). Upon infection, the enveloped virus converts its RNA genome into DNA via reverse transcription. HIV preferentially infects T-cells, macrophages, and dendritic cells. Infected T-cells ultimately die, compromising patients' immune system and leading to progression to AIDS. Without treatment, the average life expectancy of patient is 9 to 11 years following infection.

HIV is a major public health crisis worldwide. It is transmitted by bodily fluids that contain HIV with a subset of vulnerable tissues in the recipient, including the mucus membranes. Successful anti-retroviral therapy targeting HIV reverse transcriptase has made HIV infection a manageable disease, however these drugs cannot eradicate the viral load. Recently, we and others have been interested in the development of drugs that inhibit the entry of HIV into the host cell. Entry into host cell is crucial for infection and its prevention could enable more robust clearance of virus or preventative treatment of HIV.

Objective

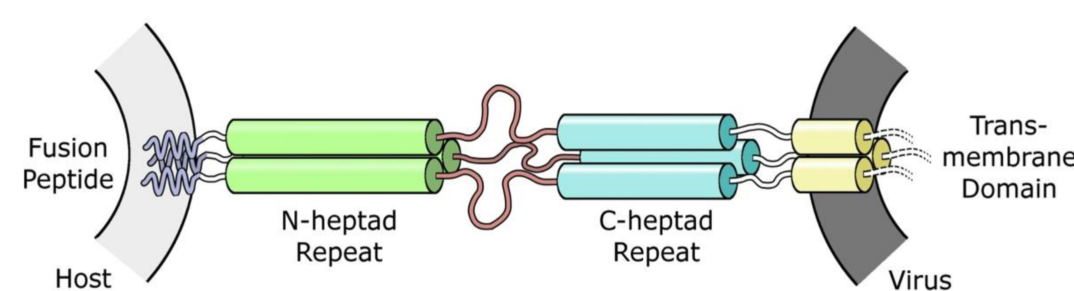
The objective of this project is to structurally characterize the binding of a variety of computationally identified compounds to the fusogenic inner helical core of gp41 using X-ray crystallography. The compounds being tested have been developed and characterized by our collaborators in the research group of Robert Rizzo at Stony Brook University. The ultimate goal is to improve the binding affinity and potency of these compounds which will require experimental structural information.

Schematic and structure of Gp41-5.



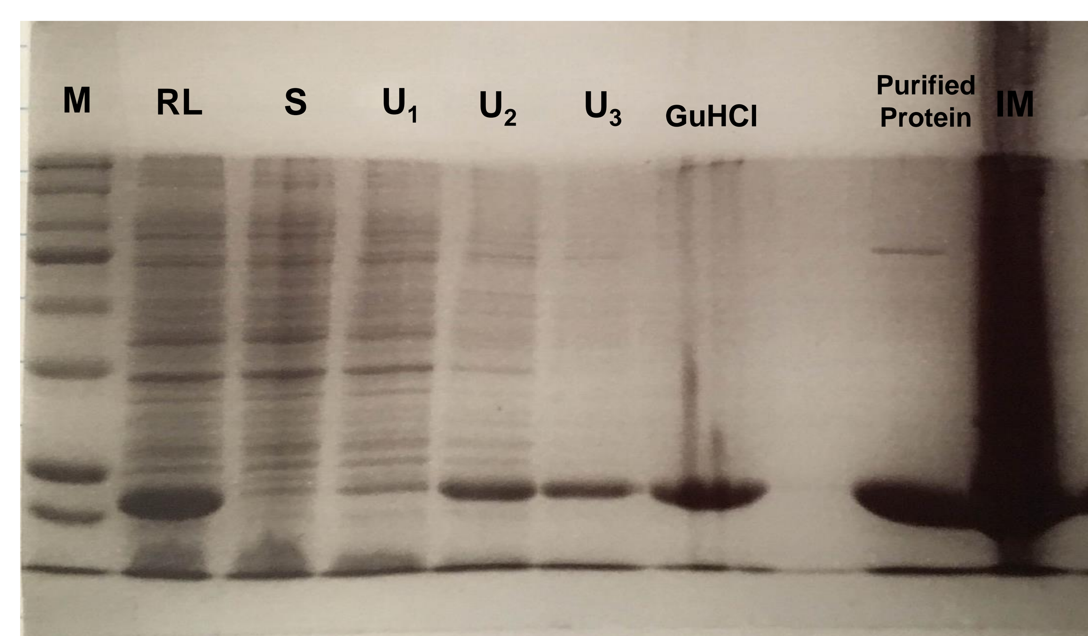
A ribbon diagram and helical cartoon of a prior crystal structure of gp41-5 at 1.5 Å resolution are shown on the left and right, respectively.[2] Gp41-5 contains five alpha helices, as compared to the six-helix bundle of the native gp41 protein. The surface of the gp41-5 five helical bundle left exposed by omitting the sixth helix mimics a pre-fusion complex and is the predicted binding site of the tested anti-fusion compounds.[3]

Role of gp41 in HIV infection



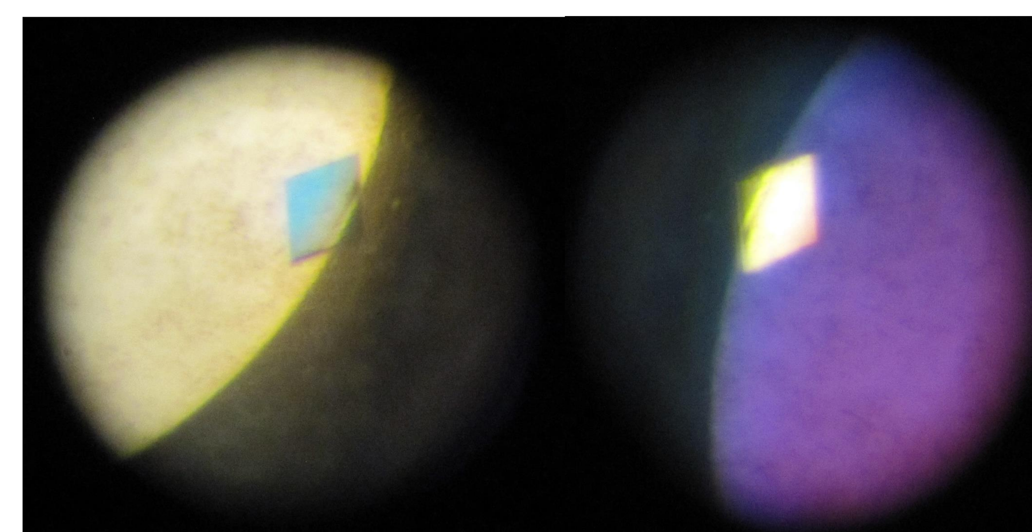
The viral protein gp41 is essential for HIV entrance into the host cell. Gp41 is a transmembrane protein that is bound to gp120. Once gp120 binds to the target cell *via* the CD40 receptor, a the formation of a helical bundle brings HIV and host cell membranes into close proximity, facilitating fusion and enabling HIV to enter the host cell.

Purification of gp41-5 protein



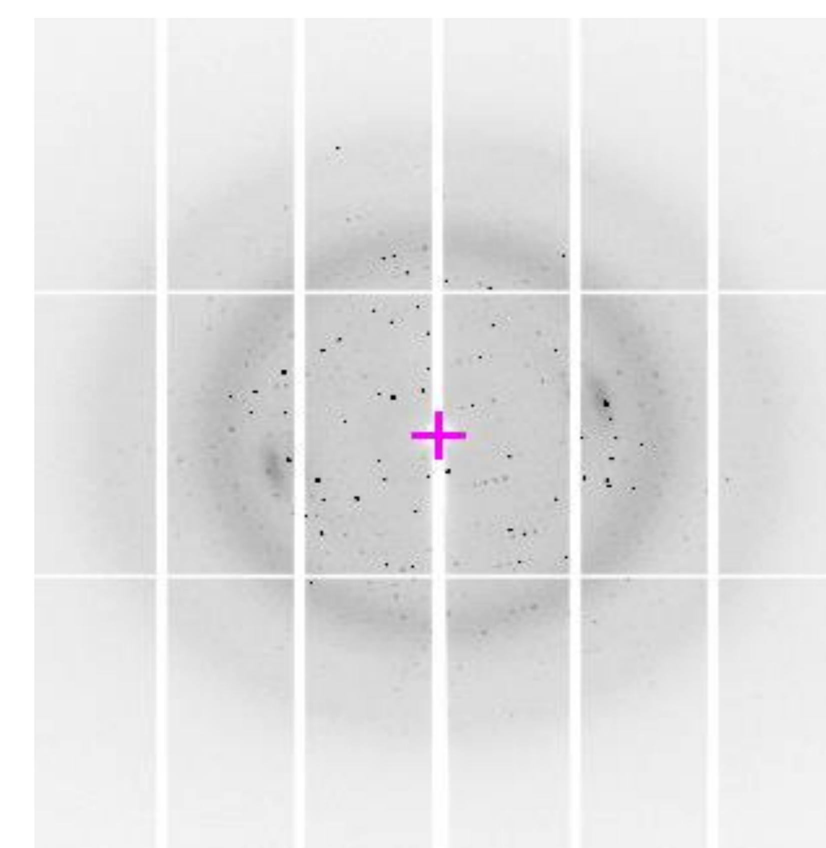
A SDS-PAGE gel of the inclusion body purification of gp41-5 is shown with lanes marked. Raw lysate (RL), supernatant (S) ,three urea wash steps ($U_{1,2,3}$), GuHCl-solubilized protein (GuHCl), purified protein, and the insoluble material after refolding (IM). Overall, 6 mg, of the purified protein product was obtained from 1.5 L of cultured *E. coli* cells.

Images of Gp41-5 crystals



Light microscopy images taken with polarized light of gp41-5 crystals. The protein was crystallized in a 1.9M, DL-malic acid, pH=7.2. The kite-shaped crystals appeared after two-three weeks of incubation at room temperature. Composition of the crystals was confirmed by re-dissolving them and performing mass spectrometry (Dr. Javier Seravalli, RBC Mass Spectrometry Core).

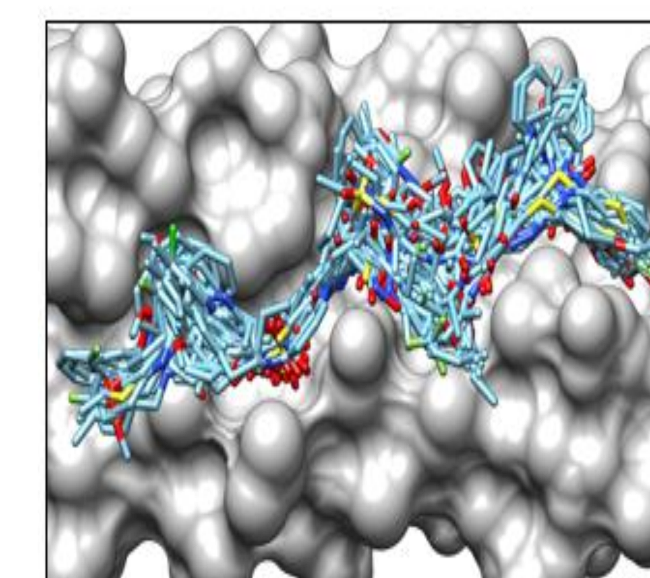
X-ray diffraction data of gp41-5 crystal



Diffraction-quality crystals of gp41-5 were obtained from sparse-matrix screening. A representative image from a 1.2 Å resolution dataset collected at SSRL 12-2 is shown from a Pilatus PAD detector.

Structure solution by molecular replacement of the crystal failed. Close analysis of the diffraction data statistics revealed a pathology in the crystal whereby non-crystallographic translational symmetry interacting with pseudosymmetry in the lattice resulted in a phenomenon called an order-disorder (or lattice translocation) defect. We have verified that the crystals contained the target protein by mass spectrometry and consulted several outside crystallographers to verify that the data are not solvable.

Compounds that act to inhibit protein fusion



High throughput computational screening has identified several lead compounds that are predicted to bind the pre-fusion exposed patch on gp41 from an initial pool of $1.4x10^6$ trial compounds. These compounds have been experimentally verified to bind and decrease viral infectivity (Dr. Amy Jacobs, SUNY Buffalo, unpublished).

Conclusion

We have successfully developed an alternative purification protocol for gp41-5 and obtained crystals of the protein, however an exotic crystal lattice defect prevents further use of these crystals. We are currently optimizing protein purification and screening for additional conditions that will deliver suitable crystals. We will be working closely with our computational collaborators in the Rizzo lab at Stony Brook University to use the structural results to improve compound design and increase binding affinity. The ultimate goal of this work is the development of novel drug candidates for the prevention and treatment of HIV infection.

Future Directions

- Improve protein yield by optimizing purification and refolding protocols
- Verify refolding by circular dichroism (CD) spectroscopy
- Re-screen crystallization conditions for alternative crystal morphologies that are free of the lattice defect
- Soak in or co-crystallize computationally identified lead compounds and determine their structures

Acknowledgements

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