NMR spectroscopy enables us to study the three-dimensional structure of proteins in aqueous solutions close to physiological conditions. By using NMR spectroscopy, it is possible to study interactions between proteins and their ligands, hormones and their receptors and antibodies and their target antigens. While in earlier days only small proteins could be studied by NMR, today larger and larger proteins can be examined and the structure of proteins up to 40 KDa can be determined. In addition to structure determination, NMR has a unique ability to study the dynamics of proteins in solution.

The main focus of our laboratory is the envelope glycoprotein of HIV-1 which is made of two subunits gp120 and gp41. These molecules form a trimeric structure consisting of three gp120 and three gp41 molecules that associate by non covalent interactions. The extracellular protein gp120 enables the binding of the virus to target cells by using a primary receptor and a co-receptor molecule found on these cells. The gp41 transmembrane protein mediates the fusion of the virus with the target cell.

HIV-1 mutates at a very high rate and as a result there is almost infinite number of HIV-1 strains. There are two major phenotypes of HIV-1: R5 viruses use the chemokine receptor CCR5 as a co-receptor and infect mostly macrophages, while X4 viruses use the CXCR4 chemokine receptor as a co-receptor and infect mostly T-cells. Some viruses are dual tropic and can use either CCR5 or CXCR4. These viruses are termed R5/X4 viruses. The sequence of the third variable (V3) region of gp120 determines the tropism of HIV-1. A single amino acid mutation at position 322 from a negatively to a positively charged residue has been found to be sufficient to switch an R5 virus to an X4 virus.

We study the structure of the V3 region using HIV-1 neutralizing antibodies generated against native gp120 and that are directed against V3. The structure of V3 peptides in complex with the Fv fragment of two such antibodies was determined by NMR spectroscopy. We assume that upon binding to the HIV-1 neutralizing antibodies the flexible V3 peptide adopt the native V3 conformation against which these antibodies were elicited.

The two antibodies that we have been studying are 0.5β and 447-52D. 0.5β is a mouse monoclonal antibody elicited against gp120 of HIV-1 IIIB and neutralizes specifically this strain of the virus. 447-52D is a human monoclonal antibody isolated from an HIV-1 strain.

Fig. 1

Space-filling representation of V3 peptides bound to HIV-1-neutralizing antibodies. Views showing the surface presented by the N-terminal beta-strand of (A), a V3

\[ \text{IIIB} \] peptide bound to 447-52D Fv (Rosen et al., 2005), (B) V3

\[ \text{IIIB} \] bound to 0.5β Fv (Tugarinov et al., 2000) and (C) V3

\[ \text{MN} \] bound to 447-52D Fv (Sharon et al., 2003).
infected donor and the exact strain of the virus that elicited its production is unknown. Unlike 0.5β the antibody 447-52D neutralizes a broad spectrum of clade-B HIV-1 strains.

The NMR studies revealed for the first time that the V3 region forms a β-hairpin. A β-hairpin conformation was found in four different complexes of antibody Fv and V3 peptides. Despite the overall similarity of four V3 structures they differ in the pairing of the residues in the β-hairpin, the side-chain orientation of the N-terminal strand residues and the network of hydrogen bonds (Rosen et al., 2005; Rosen et al., 2006; Sharon et al., 2003; Sharon et al., 2005; Tugarinov et al., 1999; Tugarinov et al., 2000). We suggested that the N-terminal strand conformation recognized by 0.5β and 447-52D represent the X4 and R5 conformations of the V3, respectively.

The Molecular Basis for Broad Neutralization by the antibody 447-52D

To understand the molecular basis underlying the breadth of HIV-1 neutralization by the antibody 447-52D we determined the structure of two different V3 peptides, V3\textsubscript{IIIb} and V3\textsubscript{IIIb}, bound to 447-52D Fv and compared these structures to that of the V3\textsubscript{IIIb} peptide bound to 0.5β antibody. We found that predominant interactions of 447-52D with three conserved residues of the N-terminal side of the V3 loop, K312-I314-I316, can account for the broad cross reactivity of 447-52D, whereas the predominant interactions of 0.5β with the variable residues R315 and Q317 underlie the strain specificity of this antibody (Rosen et al., 2005).

Cross-Strand Electrostatic Interactions at the Base of the β-Hairpin of HIV-1 V3 Region Create a Molecular Switch for Phenotype Conversion

To further investigate the influence of the amino acid sequence on the conformation of the V3, we determined the structure of the V3 region of the R5 HIV-1 strain, JR-FL, in complex with 447-52D Fv. This structure is the longest of all V3 structures determined in our laboratory and for the first time includes residues R304 and E322 at the base of the β-hairpin. These two oppositely charged residues were found to be juxtaposed in the β-hairpin structure, enabling favorable electrostatic interactions that can stabilize the R5 conformation. Comparison of the R5 conformation with the postulated X4 conformation of the V3 region (positively charged residue at position 322), reveals that electrostatic repulsion between residue 322 and 304 in X4 strains triggers the observed one register shift in the N-terminal strand of the V3 region. Hence, it is concluded that electrostatic interactions at the base of the V3 region are important for the modulation of the V3 conformation and can cause the phenotype switch (Rosen et al., 2006).

The 2F5 Epitope Is Helical in the HIV-1 Entry Inhibitor T-20

The HIV-1 envelope glycoprotein gp41 is responsible for viral fusion with the host cell. The fusion process, as well as the full structure of gp41, is not completely understood. One of the strongest inhibitors of HIV-1 fusion is a 36-residue peptide (D\textsubscript{36}-E\textsubscript{37}-gp41) named T-20, is now being used as an anti-HIV-1 drug. This peptide also contains the immunogenic sequences that represent the full or partial recognition epitope for the broadly neutralizing human monoclonal antibodies 2F5 and 4E10, respectively. Due to its hydrophobicity, T-20 tends to aggregate at high concentrations in water, and therefore the structure of this molecule in aqueous solution has not been previously determined. We expressed a uniformly \textsuperscript{15}N-labeled 42-residue peptide NN-T-20-NITN \textsuperscript{636-677}gp41 and used heteronuclear 2D and 3D NMR methods to determine its structure. Due to the additional native hydrophilic residues of gp41, NN-T-20-NITN dissolved in water, enabling for the first time determination of its secondary structure at near physiological conditions. Our results show that the NN-T-20-NITN peptide is composed of a mostly unstructured N-terminal region and a helical region beginning at the center of T-20 and extending toward the C-terminus. The helical region is found under various conditions and has been observed also in a 13-residue peptide 608-620gp41 (Biron et al., 2002). We suggest that this helical conformation is maintained in most of the different tertiary structures of the gp41 envelope protein that form during the process of viral fusion. Accordingly, an important element of the immunogenicity of gp41 and the inhibitory properties of Fuzeon may be the propensity of specific sequences in these polypeptides to assume helical structures (Biron et al., 2005).

Selected publications


Biron, Z., Khare, S., Samson, A.O., Hayek, Y., Naider, F. and

![Fig. 2](image-url)


**Acknowledgements**

We thank Dr. Fred Naider from the College of Staten Island of CUNY and Dr. Susan Zolla-Pazner from New-York University for a most fruitful collaboration. This study was supported by NIH grant GM53329.