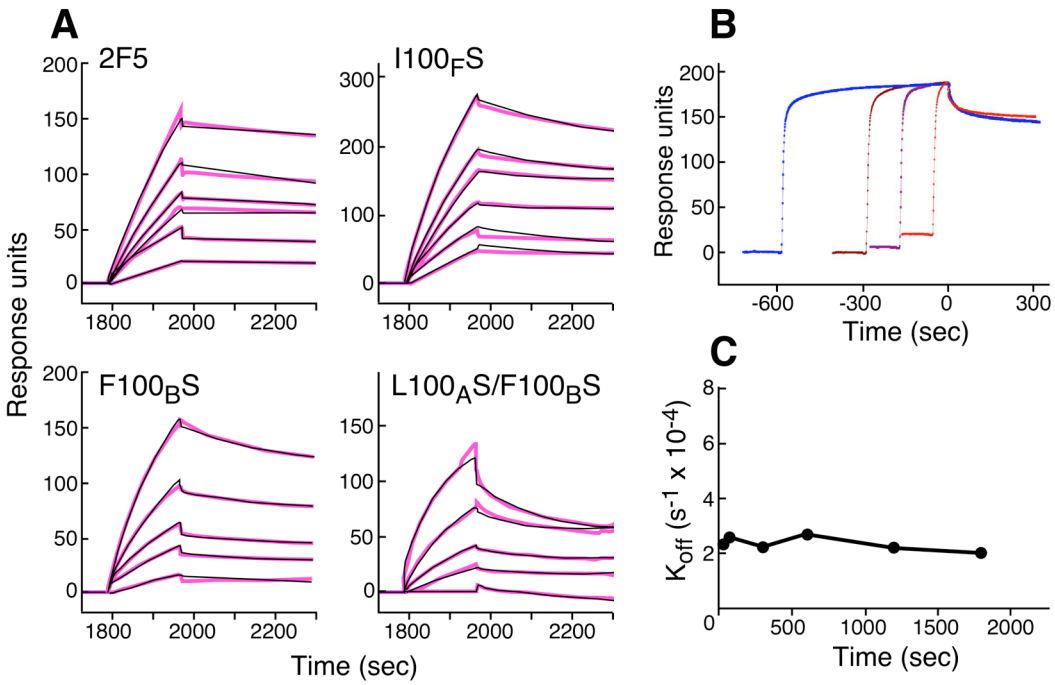
Supplementary Information

ANTIBODY MECHANICS ON A MEMBRANE-BOUND HIV SEGMENT ESSENTIAL FOR GP41-TARGETED VIRAL NEUTRALIZATION

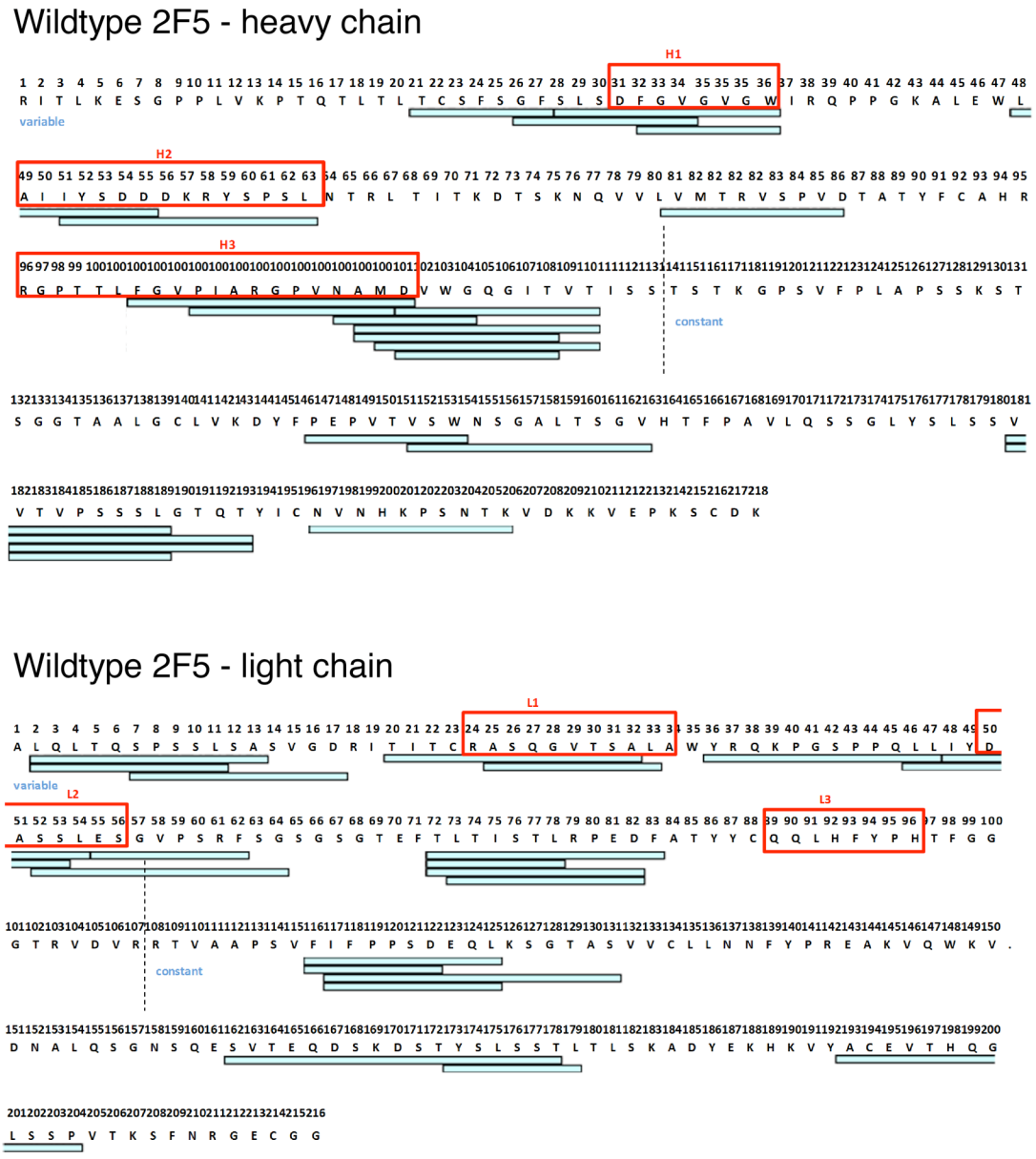
Mikyung Kim [1,2], Zhen-Yu J. Sun[3], Kasper D. Rand[4], Xiaomeng Shi[4], Likai Song[2, 5, 6], Yuxing Cheng[1, 7], Amr F. Fahmy[3], Shreoshi Majumdar[1], Gilad Ofek[8], Yongping Yang[8], Peter D. Kwong[8], Jia-huai Wang[1,2,9], John R. Engen[4], Gerhard Wagner[3] and Ellis L. Reinherz[1,2, 5]

[1]Laboratory of Immunobiology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA. [2]Department of Medicine, Harvard Medical School, Boston, MA 02115, USA. [3]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA. [4]Department of Chemistry & Chemical Biology and The Barnett Institute of Chemical & Biological Analysis, Northeastern University, Boston, MA 02115, USA. [5]Cancer Vaccine Center, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA. [6]National High Magnetic Field Laboratory, Tallahassee, FL 32310, USA. [7]PhD Program in Biological Sciences in Public Health, Harvard School of Public Health, Boston, MA 02115, USA. [8]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA. [9]Department of Pediatrics, Harvard Medical School, Boston, MA 02115, USA. Address correspondence to Dr. Ellis L. Reinherz, Laboratory of Immunobiology, Dana-Farber Cancer Institute, 77 Avenue Louis Pasteur, Boston, MA 02115; ellis_reinherz@dfci.harvard.edu;

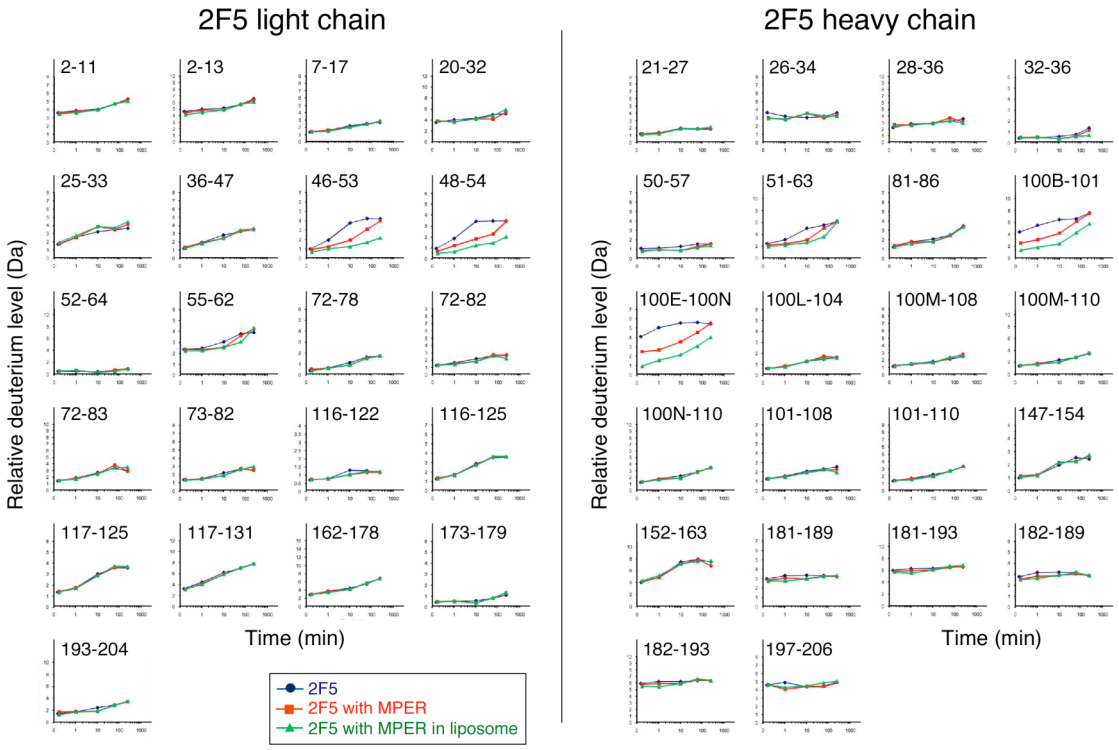
Running title: *Coordinate MPER and BNAb paratope conformational change*



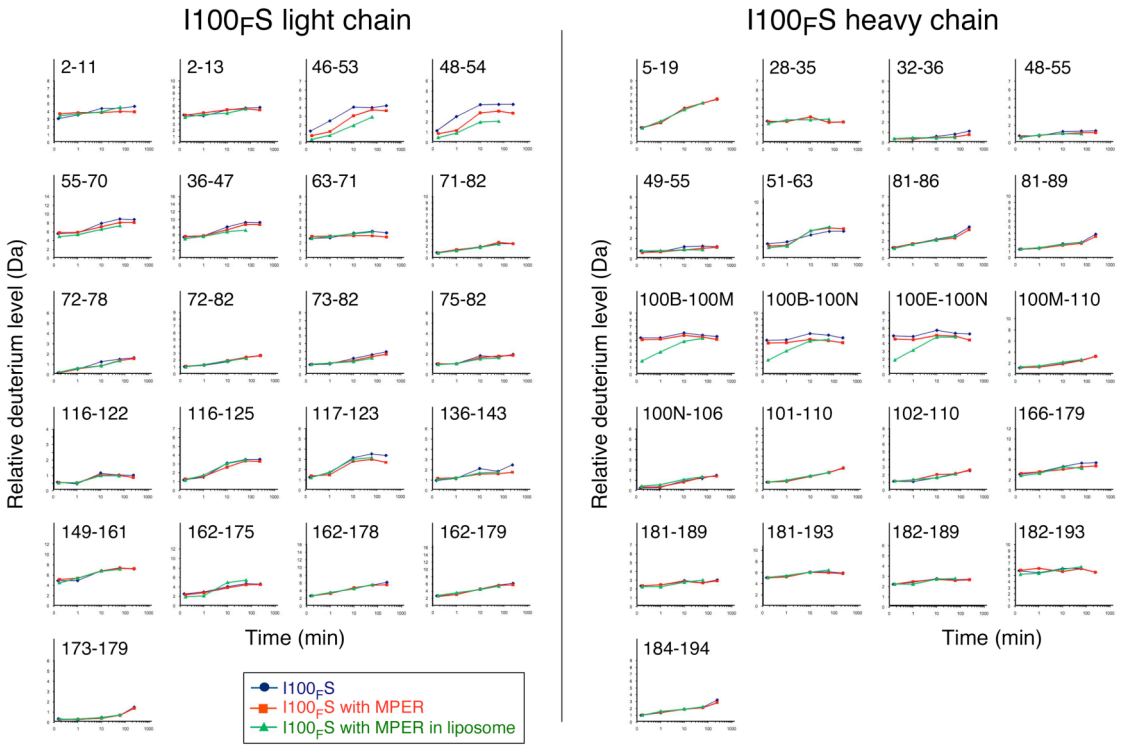
Supplementary Figure 1. Binding of 2F5 and variants to MPER/liposome complex. A) A representative binding profiles of wt 2F5 and $2F5_m$ variant mAbs to the MPER segment (red), overlaid with fits of the data (black). The MPER segment is presented on the surface of DOPC/DOPG membrane non-covalently attached to L1 chip. Each mAb was injected at varying concentrations ranging from 2 nM to 40 nM (wt 2F5); 6.6 nM to 46 nM ($I100_FS$); 23 nM to 260 nM ($F100_BS$) and 66 nM to 1 μM ($L100_AS/F100_BS$). Chi^2 values were calculated to be 8.74 for wt 2F5; 7.69 for $I100_FS$; 1.23 for $F100_BS$ and 3.3 for $L100_AS/F100_BS$. B) Effects of varying contact time on dissociation rates. Representative sensogram overlays of 660 nM 2F5 binding to the MPER/liposome complex with four different association times (from 1min to 15min) are shown. C) Plot of off-rate vs. association time. The off-rate values were from global analysis fits to binding curves, where a standard error is less than 5% in all cases. The start of dissociation was aligned on the x-axis at time zero.



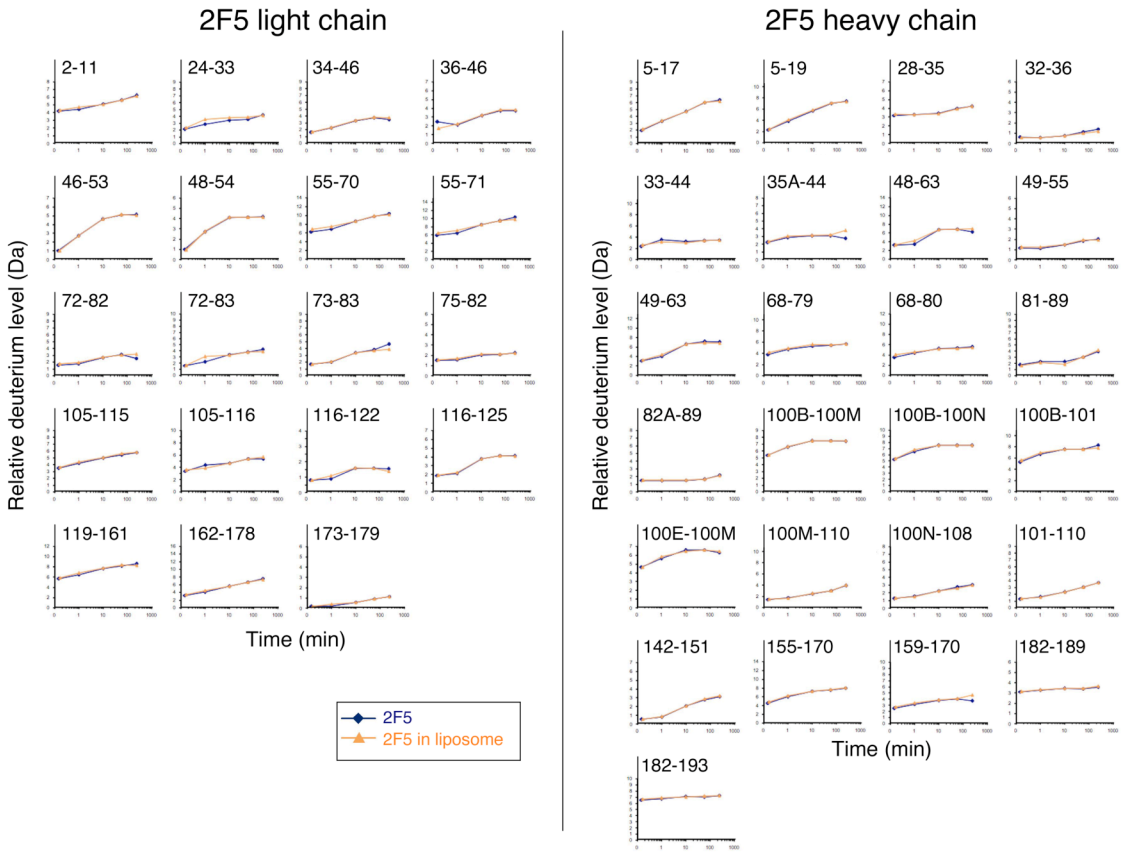
Supplementary Figure 2. Maps of the peptides produced from pepsin digestion and followed during HX-MS experiments. Representative results for digestion of wt 2F5 are shown for both heavy and light chains. Each blue bar under the sequence represents a peptide that was identified and followed. The CDR regions (Heavy chain: H1, H2, H3; Light chain: L1, L2, L3) are indicated by red boxes and the division between the variable and constant portion is indicated. Amino acid numbering is according to Ofek et al. [1]



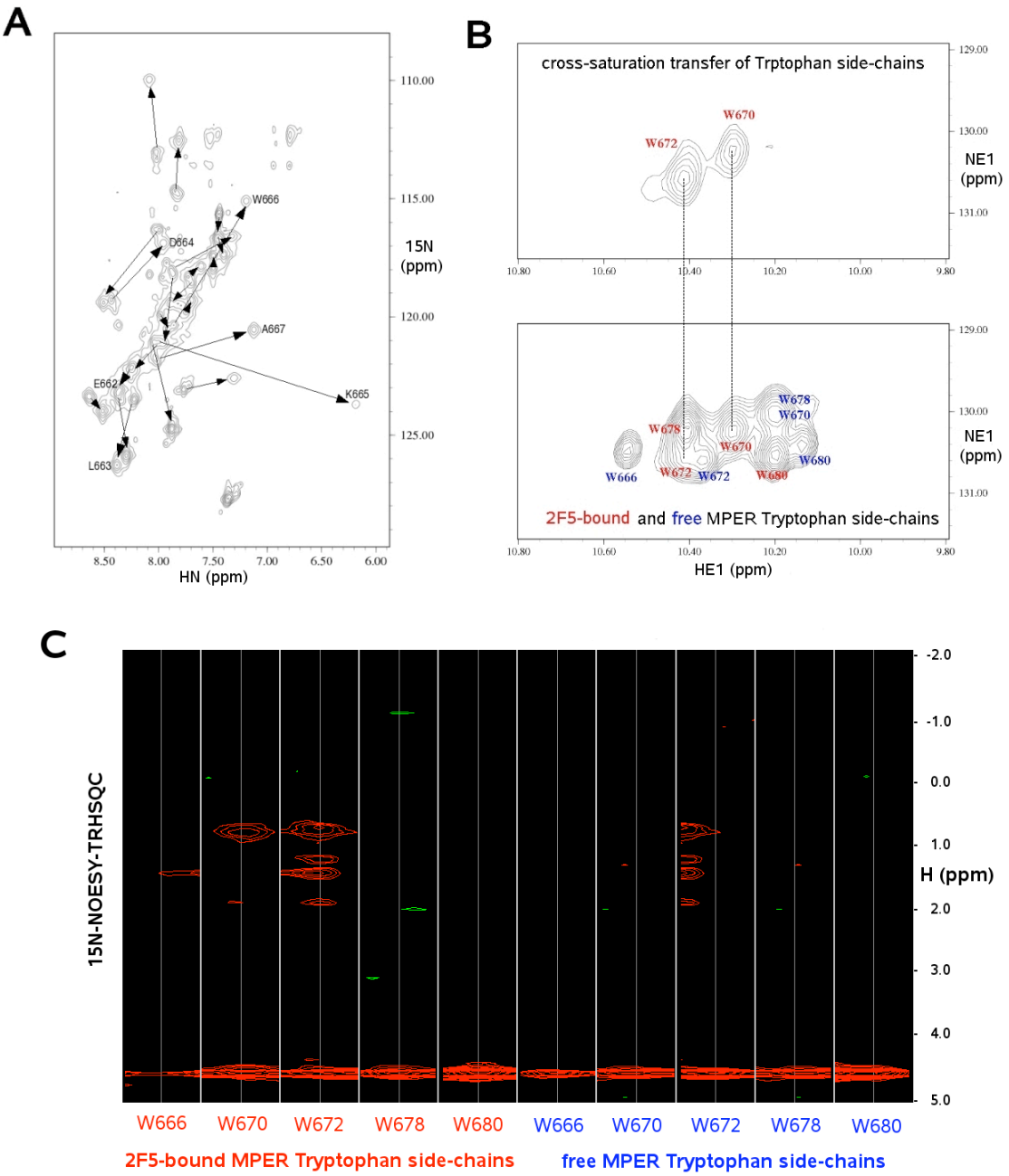
Supplementary Figure 3. The complete set of deuterium uptake curves for wt 2F5. In each graph, curves of the protein alone (blue), protein in presence of MPER (red) and protein in presence of MPER embedded in liposomes (green) are shown. The sequence number of each peptide is indicated and corresponds to Supplementary Fig. 2. The error of each data point is, at most, +/- 0.25 Da as described in Experimental Procedures. Relative deuterium level and exchange time (in minutes) are indicated on ordinate and abscissa, respectively, as in Fig. 2.



Supplementary Figure 4. The complete set of deuterium uptake curves for $2F5_m$ $I100_FS$. In each graph, the protein alone (blue), protein in presence of MPER (red) and protein in presence of MPER embedded in liposomes (green) are shown. The error of each data point is, at most, +/- 0.25 Da as described in Experimental Procedures.

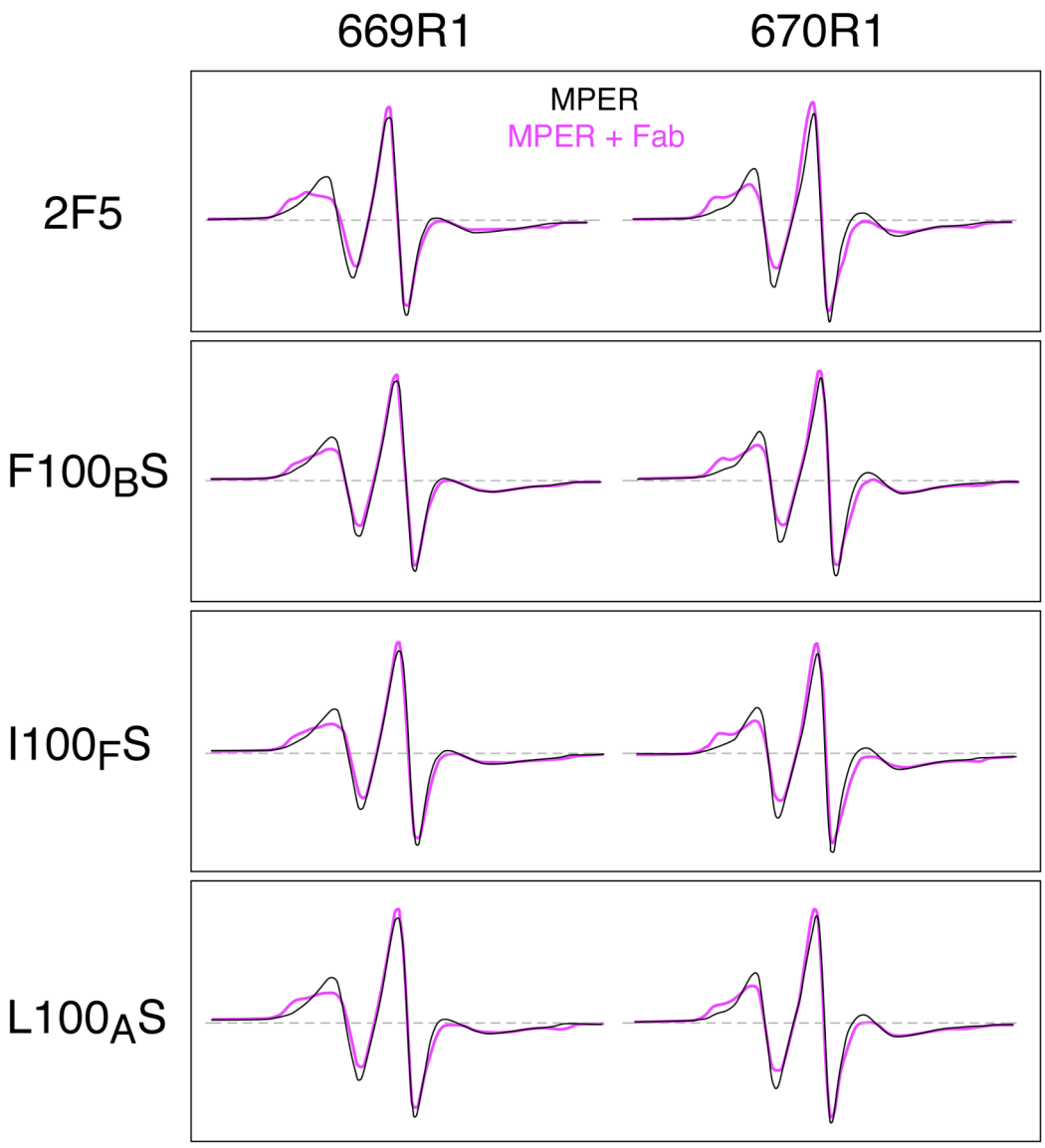


Supplementary Figure 5. The complete set of deuterium uptake curves for uncomplexed wt 2F5 or wt 2F5 mixed with liposomes. Curves in blue indicate wt 2F5 alone and curves in red indicate wt mixed with 2F5 liposome in the absence of MPER. The error of each data point is, at most, +/- 0.25 Da as described in Experimental Procedures.



Supplementary Figure 6. NMR peak shift analysis. A) 2D ^{15}N-TROSY-HSQC spectrum of both 2F5-bound and free MPER. The residues showing most dramatic peak shifts in or near the 2F5 epitope region are labeled. B) A blow-up region (upper panel) of the difference spectrum showing signal intensity changes resulting from cross saturation transfer from 2F5 Fab to side-

chain amine groups of W670 and W672 on MPER, in comparison to the reference spectrum below. C) ^{15}N-plane strips from a 120 ms mixing time ^{15}N-separated NOESY-TROSY-HSQC data set, showing NOE peaks between 2F5 Fab protons and MPER tryptophan side-chain amines. The left group (labeled in red) is from 2F5-bound MPER peptide and the right group (labeled in blue) is from unbound MPER peptide.



Supplementary Figure 7. MPER residue mobility changes upon 2F5 Fab binding by EPR. Black traces indicate EPR spectra of R1 side chains of membrane-bound peptides in the absence 2F5 Fabs. Spectra recorded in the presence of wild-type and mutant 2F5 Fabs are identified by magenta traces.

Methods

Preparation of liposomes. Lipids were mixed in chloroform and dried as thin films under a nitrogen gas stream. To remove residual organic solvent, the lipid films were further dried by vacuum pump for ≈16 h. The lipids were resuspended in 20 mM Hepes and 150 mM KCl, pH 7.0, and subjected to 10–15 freeze–thaw cycles, followed by extrusion 15 times through 2 sheets of polycarbonate membrane with a pore size of 100 nm (Avanti Polar Lipids). The concentrations of prepared LUVs were determined by phosphate contents as described previously [2]. Vesicles with virion membrane mimic were prepared at the molar ratio 9:18:20:9:45 of dioleoylphosphatidylcholine/sphingomyelin/dioleoylphosphatidylethanolamine/dioleoylphosphatidylglycerol/cholesterol (Avanti Polar Lipids). 1-Palmitoyl-2-oleoyl phosphatidylcholine/1-palmitoyl-2-oleoyl phosphatidylglycerol (POPC/POPG) LUVs at a 4:1 molar ratio were used for power saturation measurements with MPER in the presence of 2F5 and variants for comparison with previously determined results [3,4] 1,2-dioleoyl-*sn*-glycero-3-phosphocholine/1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) (DOPC/DOPG) LUVs at a 4:1 ratio were used for BIAcore analysis.

Previous studies suggested that cholesterol might modulate the permeabilization activity and the epitope accessibility of the MPER peptides [5,6]. As a consequence, we carefully examined the effect of lipid compositions on the MPER's configuration in lipid. Our previous EPR results suggested that the immersion depths of MPER residues bound to virus membrane-mimic liposomes were essentially identical to those in POPC/POPG liposomes[3,4]. In addition, 2F5 binding to the MPER on the surface of virion mimic liposome was comparable to that on the surface of DOPC/DOPG membrane by SPR. Therefore, different lipid compositions, optimized previously for each technique to provide highly reliable results [3,4], were used in this study.

Surface plasmon resonance (SPR) measurements. BIAcore experiments were carried out with a BIAcore 3000 with the Pioneer L1 sensor chip composed of alkyl chains covalently linked to a

dextran-coated gold surface at 25°C. Data analyses were performed using BIAevaluation 3.1 software (BIAcore). The running buffer was 20 mM HEPES containing 0.15 M NaCl (pH 7.4) (HBS-N). The BIAcore instrument was cleaned extensively and left running overnight with Milli-Q water to remove trace amounts of detergent. The DOPC/DOPG liposome (30 μl, 150-250uM) was applied to the sensor chip surface at a flow rate of 3 μl/min, and the liposomes were captured on the surface of the sensor chip and provided a supported lipid bilayer. To remove any multilamellar structures from the lipid surface, sodium hydroxide (20 μl, 25 mM) was injected at a flow rate of 100 μl/min, which resulted in a stable baseline corresponding to the immobilized liposome bilayer membrane with response units (RU) of 6000.

For epitope mapping of 2F5 and variant antibodies, MPER variant peptide solutions (0.5 μM) were prepared by dissolving in running buffer right before injection and the solution (60 μl) was injected over the lipid surface at a flow rate of 5 μl/min. Antibody solution (20 μg/ml) was passed over peptide-liposome complex for 3 min at a flow rate of 10 μl/min. The immobilized liposomes were completely removed with an injection of 40 mM CHAPS (25 μl) at a flow rate of 5 μl/min, followed by 10ul injection of NaOH (50mM)/isopropanol (6:4) at a 20 μl/mim flow rate, and each peptide injection was performed on a freshly prepared liposome surface.

For kinetics and affinity of 2F5 and variants to MPER peptide (ELDKWASLWNWFNITNWLWYIK), either peptide/liposome complex (1:500) was immobilized to the sensor chip surface with RU ranging from 4000 to 6000 or the MPER peptide (10 nM- 1 μM) was passed over liposome surface. Antibody solution as an analyte was then passed over peptide/liposome surface at the flow rate ranging from 30 μl to 70 μl/min. Scrambled MPER peptide/liposome surface was used as a negative control for subtraction.

Electron Paramagnetic Resonance (EPR). The EPR experiments were carried out as previously described [3]. HIV-1 HxB2 strain MPER peptides corresponding to residues 662-683 (ELDKWASLWNWFNITNWLWYIK) with a single cysteine substitution at 669 and 670 were

synthesized at the Tufts Peptide Synthesis Core facility (Boston, MA). For spin labeling, peptides were mixed with MTSL (1-oxyl-2,2,5,5,-tetramethylpyrroline-3-methyl)-methanethiosulfonate, Toronto Research Chemicals (Ontario, Canada)), and subsequently purified by reverse phase high pressure liquid chromatography (HPLC) using a C5 column (Sigma). EPR spectra were obtained on a Bruker EMX spectrometer with a Bruker High Sensitivity resonator at room temperature. All spectra were recorded at 2 mW incident microwave power with a field modulation of 2.0 G at 100 kHz. Power saturation measurements were performed on a loop-gap resonator (Molecular Specialties) at microwave power varied from 0.4 to 100 mW. N^2 gas is used to purge O^2 when necessary. The immersion-depths values were calculated by the ratio of accessibility value of O^2 to 50 mM nickel(II) ethylenediaminediacetic acid (NiEDDA).

Preparation of Fab 2F5 and variants from IgG. 2F5 and 2F5 variants antigen-binding fragment (Fab) was prepared as described previously[1]. Briefly, 20 mg of antibody was reduced in 100 mM dithiothreitol (1 h, 37°C) followed by alkylation in 2 mM iodoacetamide (48 h, 4°C) and then digested with endoproteinase Lys-C (4 μg; Roche Applied Sciences) in 25 mM Tris and 1 mM EDTA (pH 8.5) for 1h and 20 min at 37°C. The cleavage reaction was stopped with 1 mM TLCK (*Nα-p*-tosyl-l-lysine chloromethyl ketone) and 0.4 mM leupeptin, and the cleavage products were passed over a protein A-Sepharose column (Sigma) to remove Fc and intact IgG.

Transfection of envelope glycoprotein plasmids and Fluorescence Activated Cell Sorting (FACS) analysis. The Env gene from HIV-1 subtype B ADA strain was cloned into pcDNA6/V5-His6A after truncation of the cytoplasmic tail encoding segment and codon optimization to enhance protein expression. One day prior to transfection, 0.5 $X10^6$ 293T cells were seeded in the 6-well plate. After reaching near confluence (~80%), 293T cells were transfected with the plasmid using Fugene HD (Roche Diagnostics) employing a reagent: DNA ratio (v/w) of 3:1. 293T cells transfected with empty vector were used as negative control. 36

hours after the transfection, 293T cells were detached from the plate using 0.05% Trypsin/0.53 mM EDTA and washed with FACS buffer (5% FBS, 0.1% sodium azide in PBS). 1 x 10^6 cells/ml were incubated with primary antibodies at the concentration of 5 μg/ml for 2 hours at room temperature. Cells were washed three times with FACS buffer and then RPE-conjugated anti-human IgG (SouthernBiotech) was added to cells at the concentration of 0.1 μg/ml. After incubation on ice for 30 minutes, cells were washed three times and then resuspended in FACS buffer. The stained cells were acquired on FC500 analyzer (Beckman Coulter) and analyzed using Flowjo software (Tree Star, Inc.).

References

1. Ofek, G.*, et al.* Structure and mechanistic analysis of the anti-human immunodeficiency virus type 1 antibody 2F5 in complex with its gp41 epitope. *J Virol* **78**, 10724-10737 (2004).
2. Bottcher, C.J.F., van Gent, C.M. & Pries, C. A rapid and sensitive sub-micro phosphorus determination. *Anal Chim Acta* **24**, 203-204 (1961).
3. Song, L.*, et al.* Broadly neutralizing anti-HIV-1 antibodies disrupt a hinge-related function of gp41 at the membrane interface. *Proc Natl Acad Sci U S A* **106**, 9057-9062 (2009).
4. Sun, Z.Y.*, et al.* HIV-1 broadly neutralizing antibody extracts its epitope from a kinked gp41 ectodomain region on the viral membrane. *Immunity* **28**, 52-63 (2008).
5. Huarte, N., Lorizate, M., Kunert, R. & Nieva, J.L. Lipid modulation of membrane-bound epitope recognition and blocking by HIV-1 neutralizing antibodies. *FEBS Lett* **582**, 3798-3804 (2008).
6. Nieva, J.L., Apellaniz, B., Huarte, N. & Lorizate, M. A new paradigm in molecular recognition? Specific antibody binding to membrane-inserted HIV-1 epitopes. *J Mol Recognit* **24**, 642-646 (2011).