Supporting Information for:

Insights into the conformation of the membrane proximal regions critical to the trimerization of the HIV-1 gp41 ectodomain bound to dodecyl phosphocholine micelles

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Figure S1

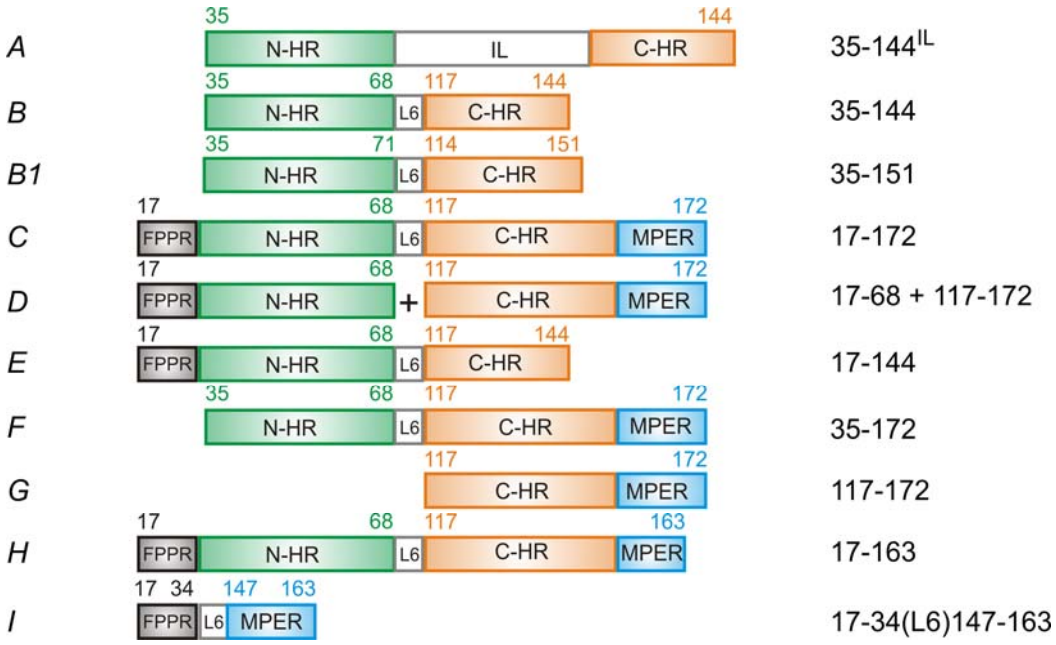

Figure S1. Various gp41 constructs used in this study and their designations. See Fig 1 for Env precursor numbering and Fig S2 for the exact sequence of each construct. Molar masses are listed in Table 1 and Fig S2.

Figure S2

A) 35-144^{IL}

Expressed with a His-tag:

GSSHHHHHHS SGLVPRGSHM SGIVQQQNNL LRAIEAQQHL LQLTVWGIKQ LQARILAVER YLKDQQLLGI WGASGKLIAT TAVPWNASWS NKSLEQIWNH TTWMEWDREI NNYTSLIHSL IEESQNQQEK

Mass (^{2}H ^{13}C-d7 ^{15}N) – 16585.2

B) 35-144

Expressed with a His-tag:

GSSHHHHHHS SGLVPRGSHM SGIVQQQNNL LRAIEAQQHL LQLTVWGIKQ LQARSGGRGG WMEWDREINN YTSLIHSLIE ESQNQQEK

Final purified protein:

GSHMSGIVQQ QNNLLRAIEA QQHLLQLTVW GIKQLQARSG GRGGWMEWDR EINNYTSLIH SLIEESQNQQ EK

Mass (H ^{12}C ^{14}N) – 8283.9

B1) 35-151

Expressed with a His-tag:

GSSHHHHHHS SGLVPRGSHM SGIVQQQNNL LRAIEAQQHL LQLTVWGIKQ LQARILASGG RGGHTTWMEW DREINNYTSL IHSLIEESQN QQEKNEQELL E

Final purified protein:

GSHMSGIVQQ QNNLLRAIEA QQHLLQLTVW GIKQLQARIL ASGGRGGHTT WMEWDREINN YTSLIHSLIE ESQNQQEKNE QELLE

Mass (^{2}H ^{13}C-d7 ^{15}N) - 10822.9

C) 17-172

Expressed and purified from inclusion bodies:

STMGAASMTL TVQARQLLSG IVQQQNNLLR AIEAQQHLLQ LTVWGIKQLQ ARSGGRGGWM EWDREINNYT SLIHSLIEES QNQQEKNEQE LLELDKWASL WNWFNITNWL WYIK

Mass (^{2}H ^{13}C-d7 ^{15}N) – 14824.7

D) 17-68 + 117-172

Expressed and purified from inclusion bodies:

STMGAASMTL TVQARQLLSG IVQQQNNLLR AIEAQQHLLQ LTVWGIKQLQ ARILASGLVP RGSGGHTTWM EWDREINNYT SLIHSLIEES QNQQEKNEQE LLELDKWASL WNWFNITNWL WYIK

Final purified sequences for trimer assembly as peptides:

17-68:

STMGAASMTL TVQARQLLSG IVQQQNNLLR AIEAQQHLLQ LTVWGIKQLQ ARILASGLVP R

Mass (^{2}H ^{13}C-d7 ^{15}N) - 7425.4

117-172:

GSGGHTTWME WDREINNYTS LIHSLIEESQ NQQEKNEQEL LELDKWASLW NWFNITNWLW YIK

Mass (^{2}H ^{13}C-d7 ^{15}N) - 8577.6

E: 17-144

Expressed and purified from inclusion bodies:

STMGAASMTL TVQARQLLSG IVQQQNNLLR AIEAQQHLLQ LTVWGIKQLQ ARSGGRGGWM EWDREINNYT SLIHSLIEES QNQQEK

Mass (^{2}H ^{13}C-d7 ^{15}N) - 10782.1

F: 35-172

Expressed and purified from inclusion bodies:

SGIVQQQNNL LRAIEAQQHL LQLTVWGIKQ LQARSGGRGG WMEWDREINN YTSLIHSLIE ESQNQQEKNE QELLELDKWA SLWNWFNITN WLWYIKGSGK KKKD

Mass (^{2}H ^{13}C-d7 ^{15}N) - 13677.5

G: 117-172

Expressed with a His-tag:

GSSHHHHHHS SGSTMGAASM TLTVQARQLL SGIVQQQNNL LRAIEAQQHL LQLTVWGIKQ LQARSGLVPR GSGGWMEWDR EINNYTSLIH SLIEESQNQQ EKNEQELLEL DKWASLWNWF NITNWLWYIK

Final purified protein:

GSGGWMEWDR EINNYTSLIH SLIEESQNQQ EKNEQELLEL DKWASLWNWF NITNWLWYIK

Mass (^{2}H ^{13}C-d7 ^{15}N) - 8204.3

H) 17-163

Expressed and purified from inclusion bodies:

STMGAASMTL TVQARQLLSG IVQQQNNLLR AIEAQQHLLQ LTVWGIKQLQ ARSGGRGGWM EWDREINNYT
SLIHSLIEES QNQQEKNEQE LLELDKWASL WNWFN

Mass (^{2}H ^{13}C-d7 ^{15}N) - 13460.4

I) 17-34(L6)147-163

Expressed with a His-tag:

GSSHHHHHHS SGLVPRGSST MGAASMTLTV QARQLLSGGR GGEQELLELD KWASLWNWFN

Final purified protein:

GSSTMGAASM TLTVQARQLL SGGRGGEQEL LELDKWASLW NWFN

Mass (H ^{13}C ^{15}N) - 5065.4

Figure S2. Amino acid sequences of gp41 constructs A through I listed in Fig S1. Underlined sequences indicate nonnative residues.

Figure S3

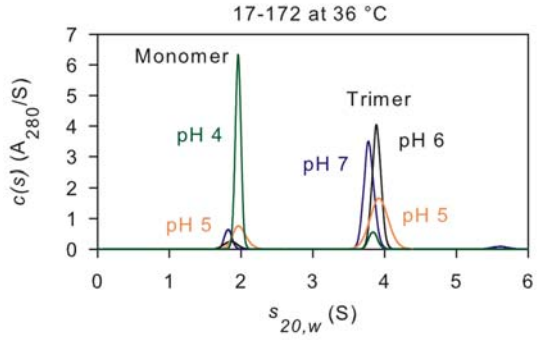

Figure S3. Sedimentation velocity absorbance *c(s)* distributions for 17-172 across pH values from 4 to 7 in the presence of excess of DPC micelles at 36 ºC. Samples were prepared exactly as described when carrying out the SV analysis at 20 ºC (see Fig 2D). For details, see Materials and Methods.

Comment [B1]: The it is rather perplexing that the trimer:monomer is ~3:1 at the higher temp, vs 1:1 at 20C

Figure S4

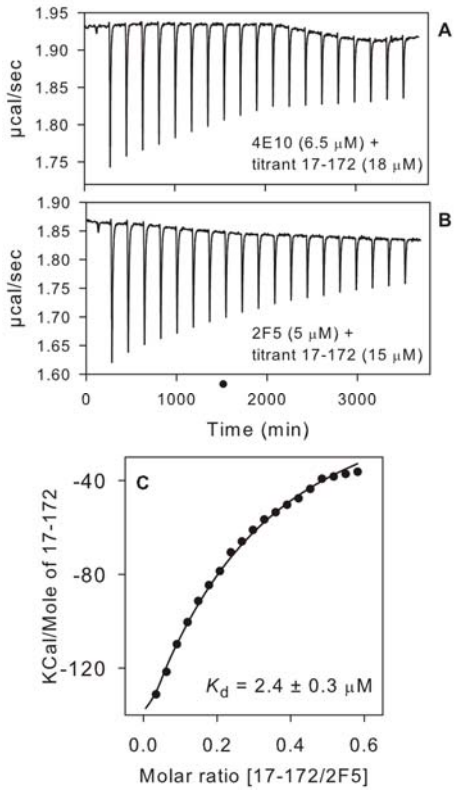

Figure S4. Binding isotherm for the interaction of 4E10 or 2F5 with 17-172 at 28 °C. (A and B) The peaks indicate the heat released after each addition of 17-172 into the antibody solution both maintained in 10 mM Tris-HCl, pH 7.6, 150 mM NaCl and 2 mM DPC. (C) The data for titration of 2F5 with 17-172 were best fit using a single binding constant to calculate the thermodynamic parameters.

Figure S5

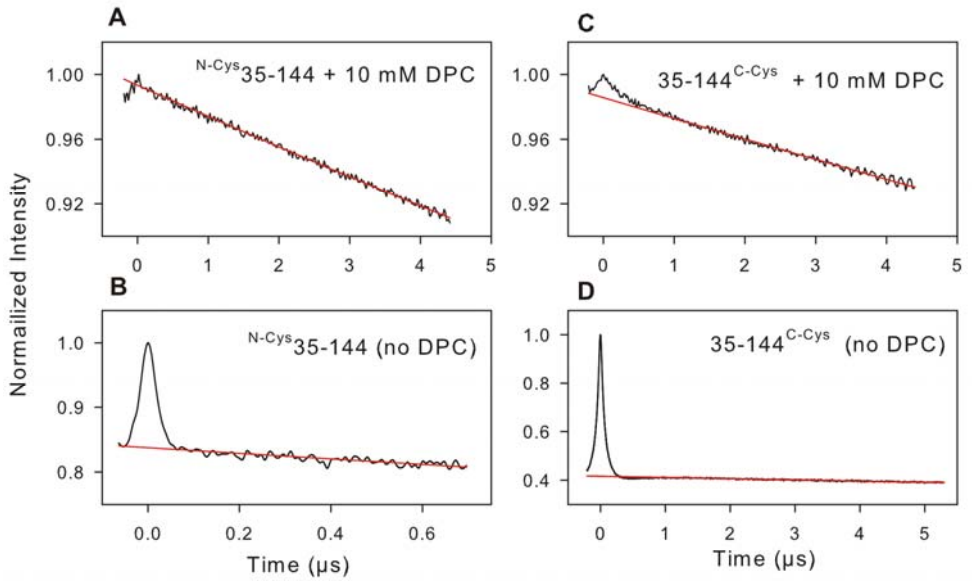

Figure S5. Raw DEER data of fully deuterated 35-144 construct bearing a deuterated nitroxide-label at the N- (A and B) or the C terminus (C and D). DEER measurement was carried out in 10 mM Tris-HCl, pH 7.6, 150 mM NaCl either in the absence (B and D) or presence (A and C) of excess molar equivalents of DPC micelles. Red traces are the exponential background functions employed to separate the random inter-molecular dipolar couplings from the desired intra-molecular dipolar couplings. The results of the DeerAnalysis2015 Tikhonov Regularization fits [1] of the background corrected data acquired in the absence of DPC are shown for both constructs (B and D). These previously published results [2] are shown here solely for the purpose of comparison with data acquired with the same constructs in the presence of DPC micelles (A and C).

Figure S6

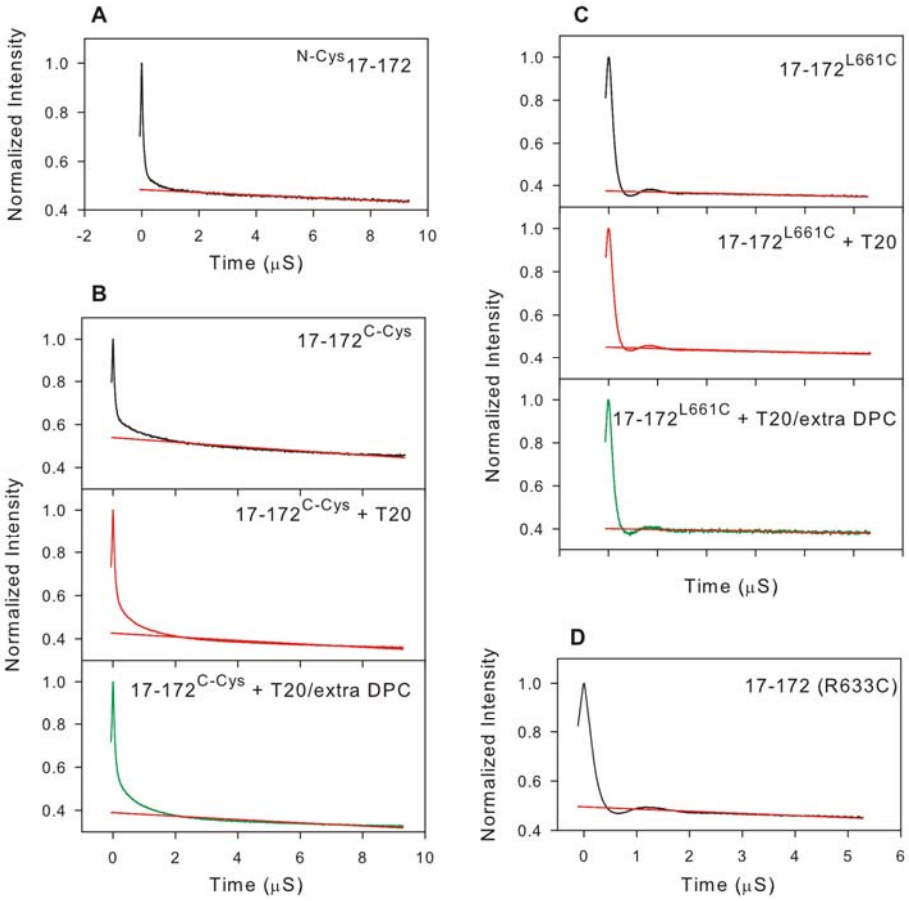

Figure S6. Raw DEER data acquired with spin labels in different positions of the 17-172 construct at pH 7 in the presence of DPC. Red traces in all plots indicate the exponential background functions employed to separate the random inter-molecular dipolar couplings from the desired intra-molecular dipolar couplings. Panels A through D match with those shown in Fig 5 (left panels).

Figure S7

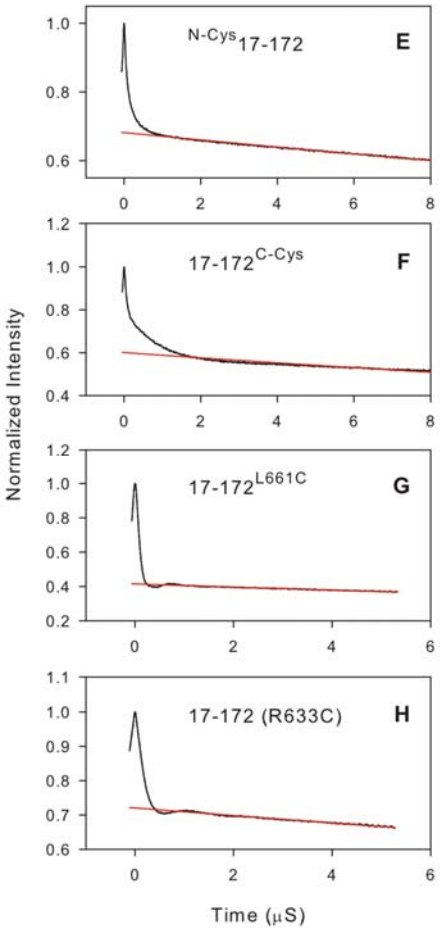

Figure S7. Raw DEER data acquired with spin labels in different positions of the 17-172 construct at pH 4 in the presence of DPC. Red traces in all plots indicate the exponential background functions employed to separate the random inter-molecular dipolar couplings from the desired intra-molecular dipolar couplings. Panel E through H match with those shown in Fig 5 (right panels).

Figure S8

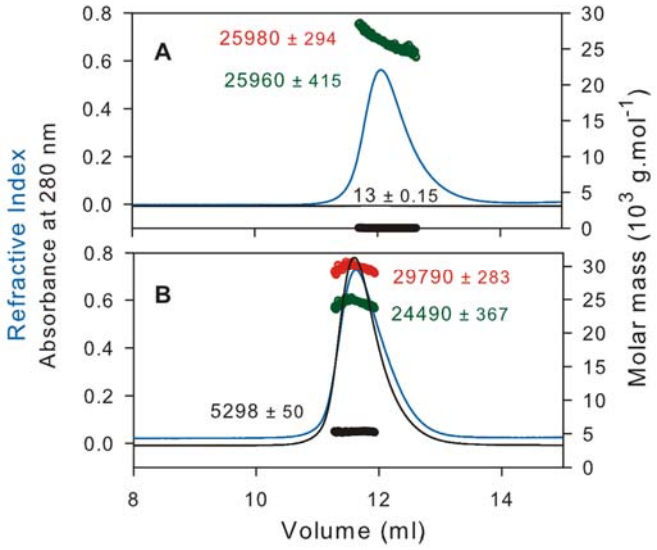

Figure S8. Molecular mass estimation of T20 bound to DPC micelles by SEC-MALS. The plots show the T20-DPC micelle composition (B) as compared to an identical injection without T20 (A). T20 (black) and DPC-micelle (green) mass contributing to the combined mass (red) of the complex are indicated beside the peak. The RI trace (blue) matches with the trace of absorbance at 280 nm (black) consistent with the higher mass of one T20 bound to a micelle (B, ~30 kDa) by eluting earlier than the DPC-micelle peak (in A, ~26 kDa). The calculated mass of T20 is 4492 Da.

References

1. Jeschke, G, Chechik, V, Ionita, P, Godt, A, Zimmermann, H, et al. (2006) DeerAnalysis2006 - a comprehensive software package for analyzing pulsed ELDOR data. Appl Magn Reson 30:473-498.

2. Louis, JM, Baber, JL, Clore, GM (2015) The C34 Peptide Fusion Inhibitor Binds to the Six-Helix Bundle Core Domain of HIV-1 gp41 by Displacement of the C-Terminal Helical Repeat Region. Biochemistry 54:6796-6805.