Supporting Information

Structural and biophysical characterizations of HIV-1 matrix trimer binding to lipid nanodiscs shed light on virus assembly

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Running Title: *Interaction of HIV-1 matrix trimer with membranes*

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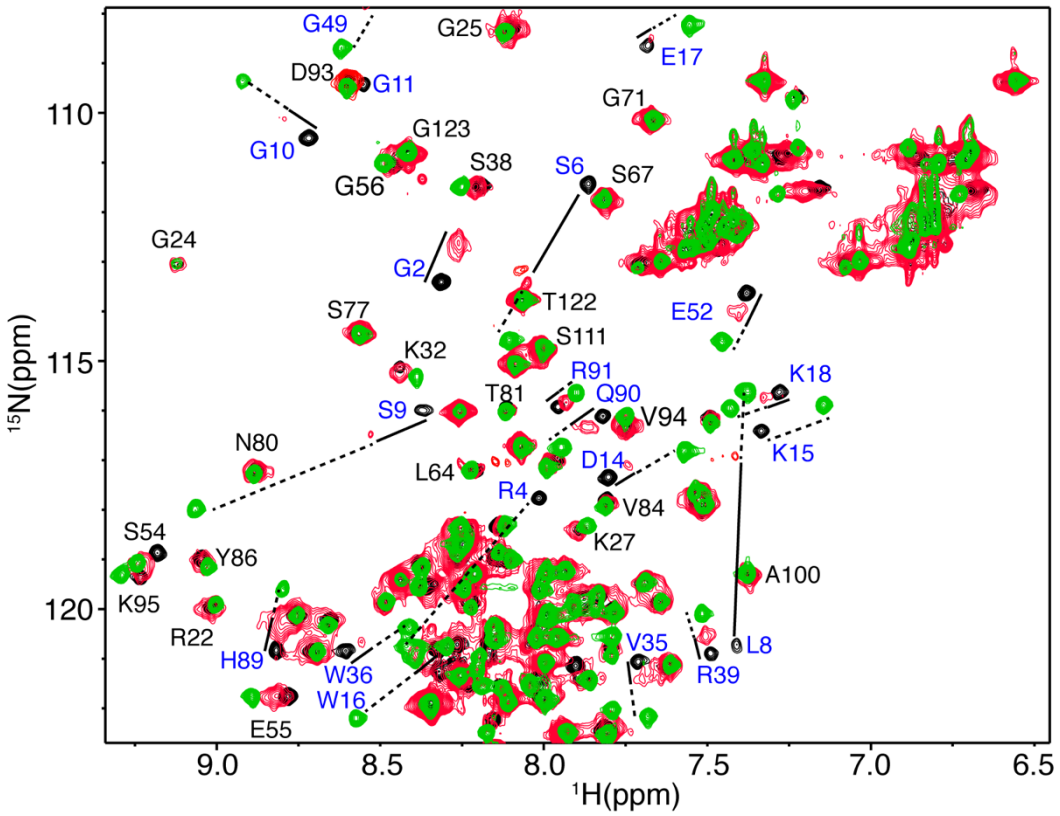

Fig. S1. NMR spectra of MA upon titration with POPC NDs. Overlay of 2D ^{1}H-^{15}N HSQC spectra for ^{15}N-labeled samples of MA (100 μM) in the absence (black) or presence of 100% POPC NDs (red) at molar ratio ND:MA of 0.3:1. Spectrum of the myr(–)MA protein is shown in green. Upon binding of POPC NDs, a subset of ^{1}H-^{15}N resonances of MA (labeled in blue) shifted dramatically toward the corresponding signals of myr(–)MA (dotted line), indicating myr exposure.

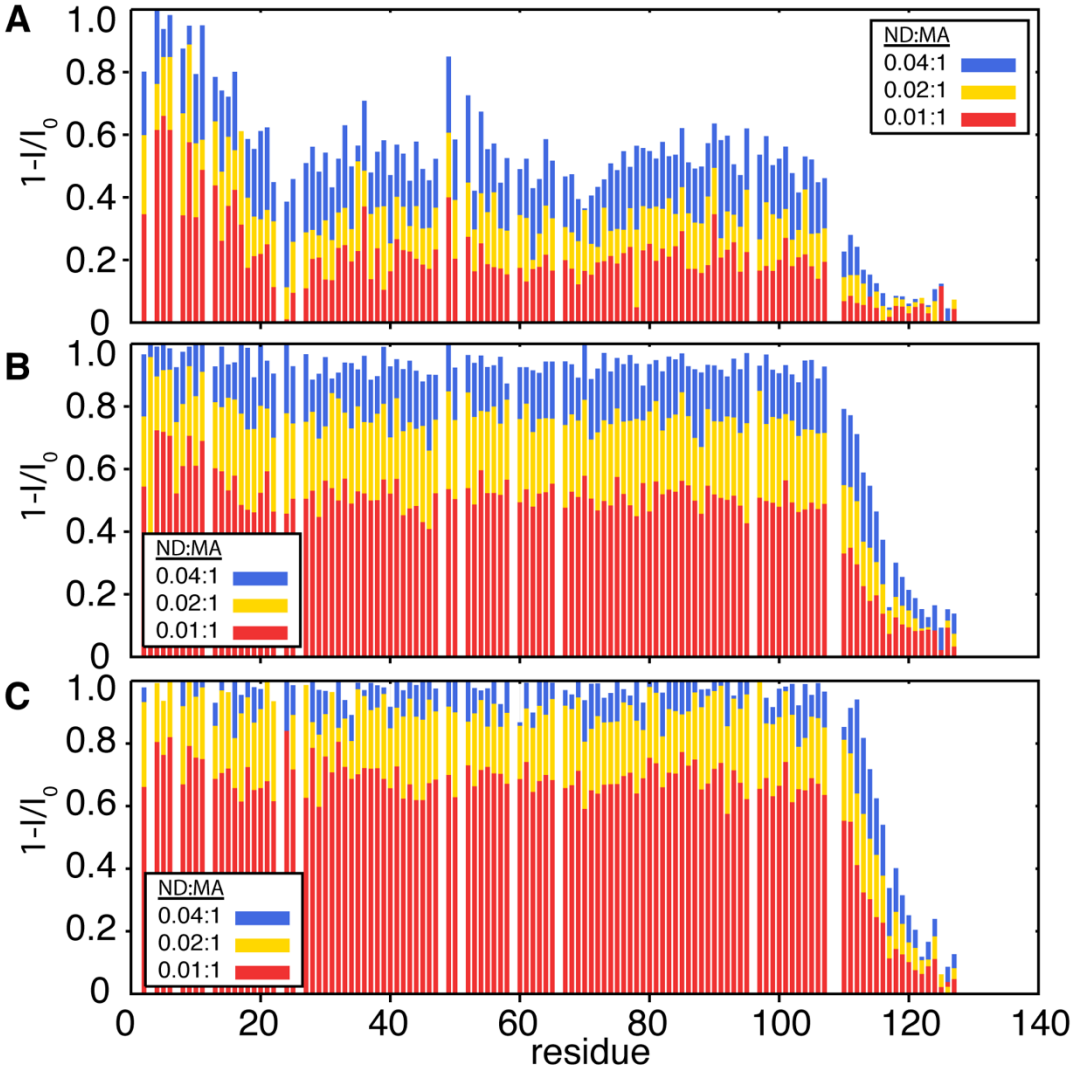

Fig. S2. Spectral analysis of NMR data of MA–ND. Histograms showing relative reduction in signal intensity (RRI) vs. residue numbers calculated from the HSQC spectra of MA upon titration with *A*, POPC:POPS (80:20); *B*, POPC:POPS (50:50); and *C*, POPC:POPS:PI(4,5)P_2 (72:20:8) NDs. RRI values were calculated by the formula = 1 - I/I_0, where (0 is no reduction, 1 meaning signal disappearance), I is the peak integral in the presence of ND, and I_0 is peak integral in the absence of a ND.

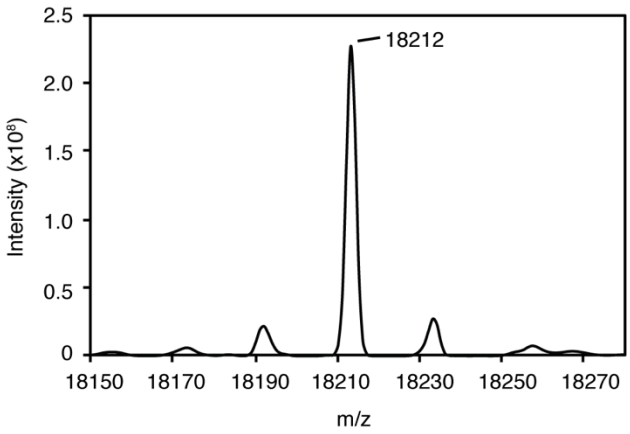

Fig. S3. Maximum entropy analysis of ESI-ToF spectrum of intact $MA_{122}FD$. The molecular mass corresponds to a myristoylated protein (theoretical mass = 18212.6 Da).

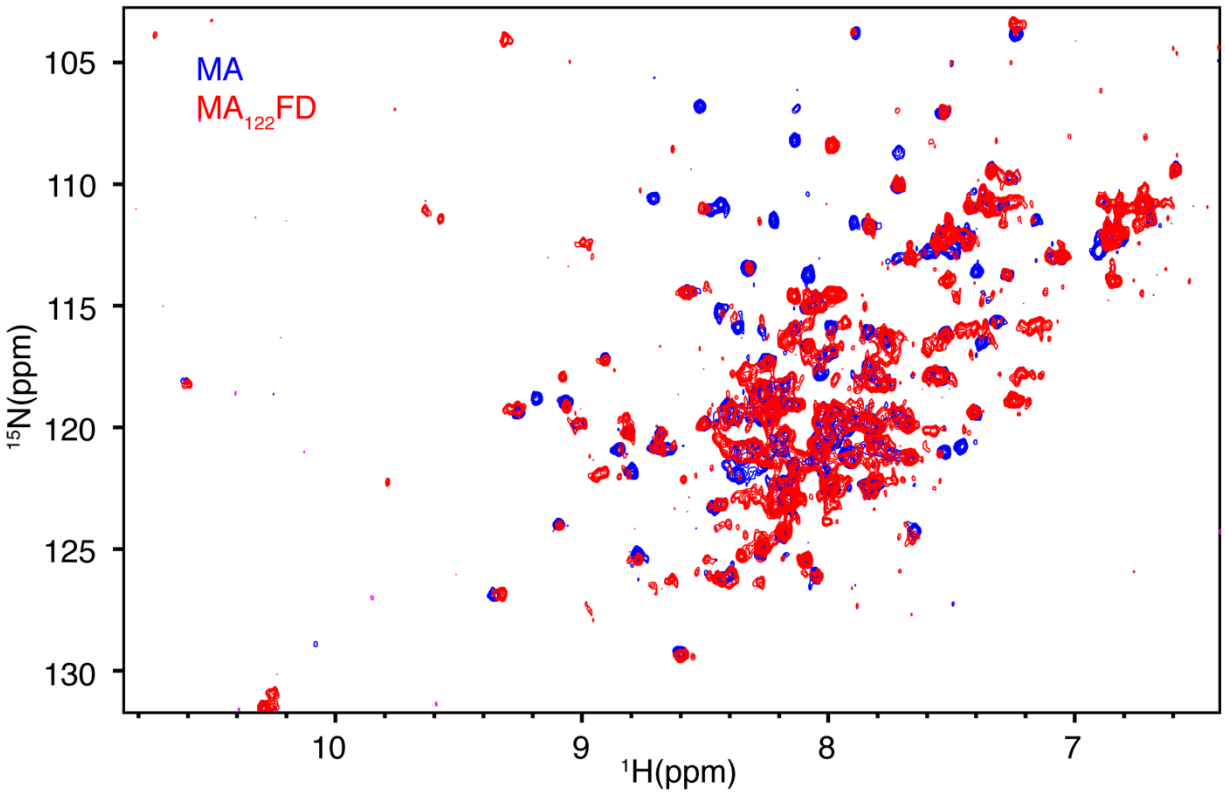

Fig. S4. An overlay of 2D ^{1}H-^{15}N NMR spectra for MA (blue) and MA$_{122}$FD (red). The similarity of the spectra indicate that the structure of MA is not altered upon fusion of the FD domain to the C-terminus of MA.

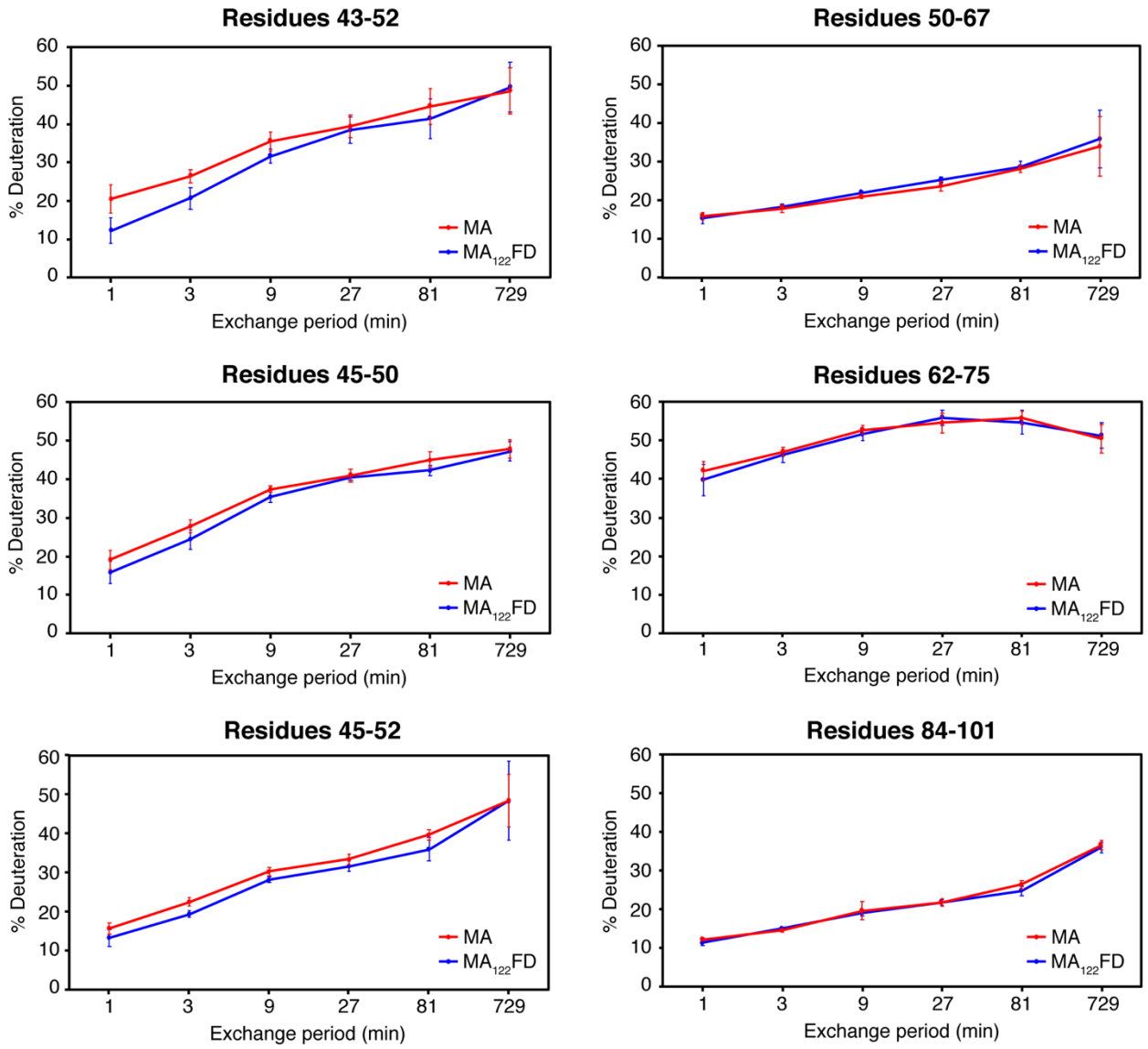

Fig. S5. HDX-MS data of MA and $MA_{122}FD$. Points and error bars represent the average values and 95% confidence intervals calculated from 6 independent HDXMS experiments for several peptide fragments of MA and $MA_{122}FD$. Left column: MA derived peptides showing protection in trimeric $MA_{122}FD$. Right Column: MA derived peptides showing no change in protection in the trimeric form.

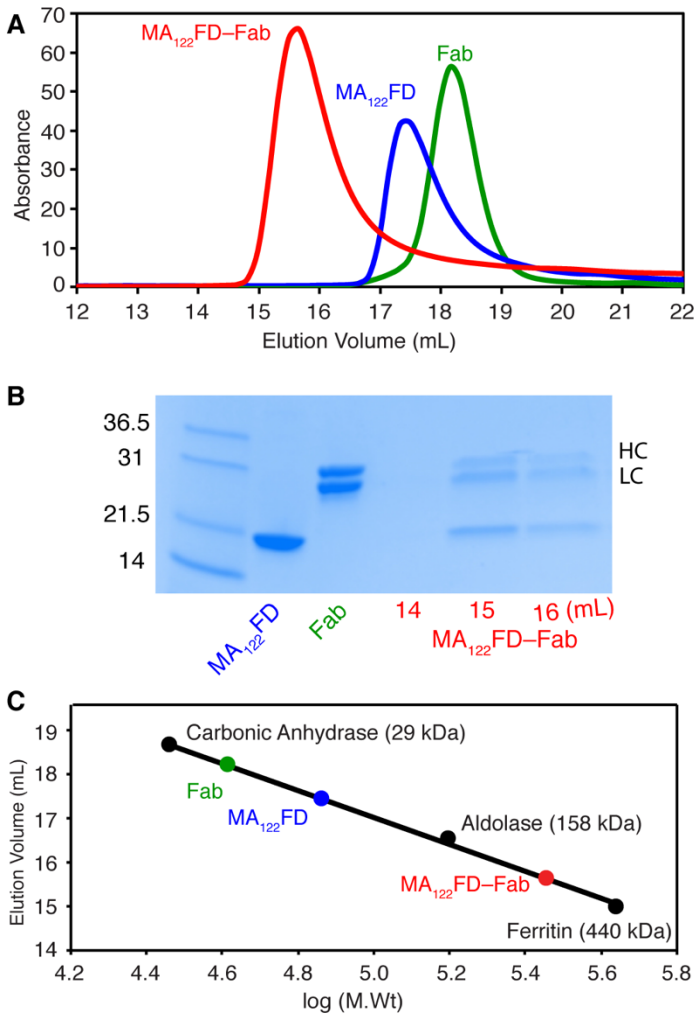

Figure S6. Gel filtration assay of the $MA_{122}FD$–Fab complex. *A*, Elution profiles of $MA_{122}FD$, Fab and their complex on a Superose 6 (10/300 GL) column. *B*, SDS-PAGE of the $MA_{122}FD$–Fab complex and Superose 6 elution fractions. *C*, A molecular weight calibration kit was used to determine the approximate molecular weight of the proteins.

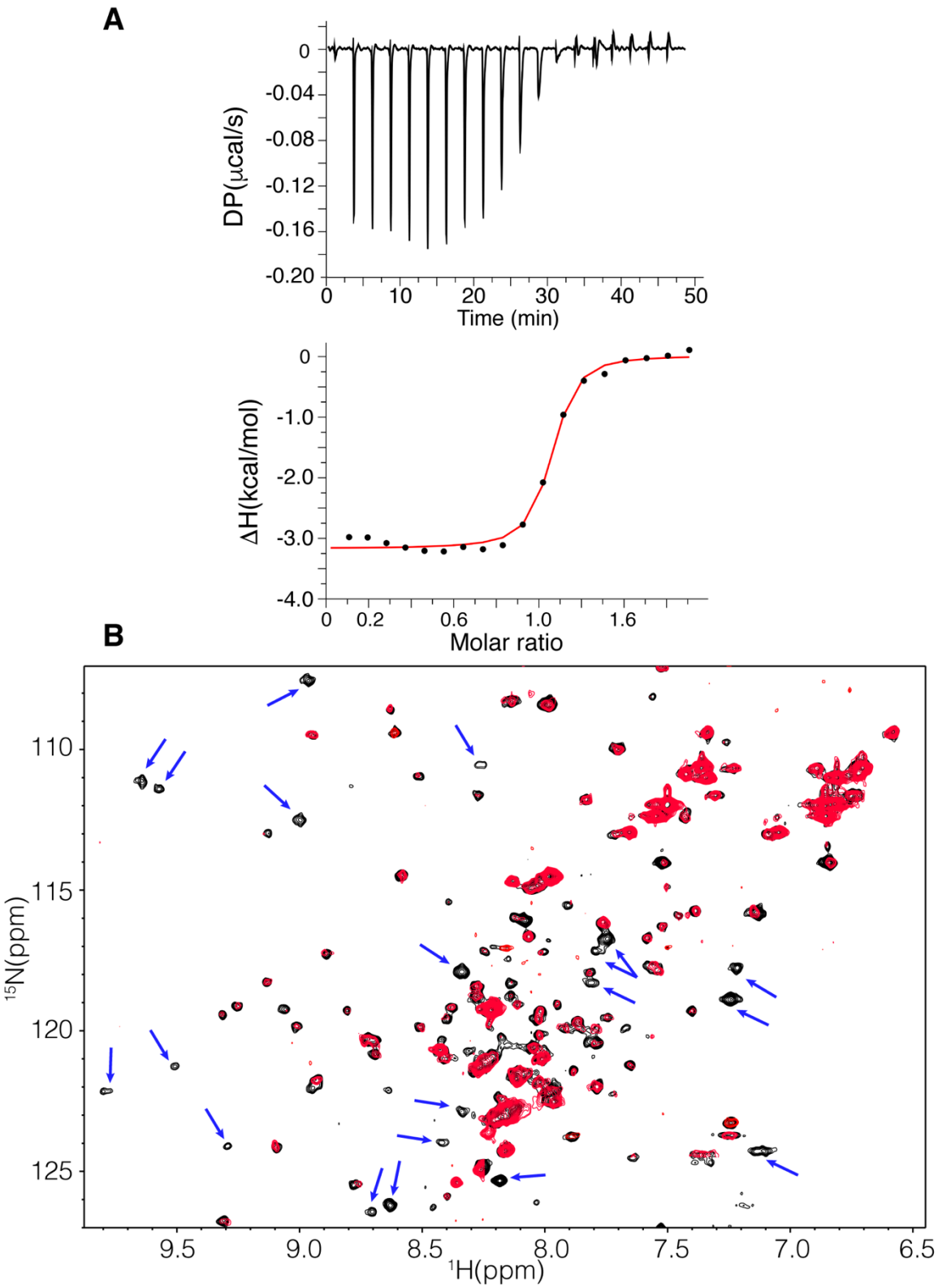

Figure S7. ITC and NMR data of Fab binding to $MA_{122}FD$. *A*, ITC data obtained for $MA_{122}FD$ (305 µM) titration into Fab (34.5 µM) in a buffer containing 50 mM phosphates (pH 6) and 50 mM NaCl. The top panel shows the baseline adjusted thermogram and the bottom panel depicts the binding isotherm and fit to the data (red line). *B*, overlay of 2D ^{1}H-^{15}N HSQC spectra of 70 µM myr(–)$MA_{122}FD$ in the free (black) and Fab-bound (red) states. The nearly identical shifts for MA signals indicate that Fab binding to the FD fragment does not alter the structure of MA. Blue arrows denote FD signals that are shifted by Fab binding.