Supporting Information For

Dynamic Regulation of HIV-1 Capsid Interaction with the Restriction Factor TRIM5α - Magic Angle Spinning NMR and MD Simulations

Caitlin M Quinn[1,2#], Mingzhang Wang[1,2#], Matthew P. Fritz[1,2], Brent Runge[1,2], Jinwoo Ahn[2,3], Chaoyi Xu[1], Juan R. Perilla[1,2*], Angela M. Gronenborn[2,3*], and Tatyana Polenova[1,2*]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh, Pittsburgh, PA 15260; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260

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[#]These authors contributed equally

***Corresponding authors:** Tatyana Polenova, Department of Chemistry and Biochemistry, University of Delaware, Newark, DE, USA, Tel.: (302) 831-1968, Email: tpolenov@udel.edu; Angela M. Gronenborn, Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15260, USA, Tel.: (412) 648-9959; Email: amg100@pitt.edu; Juan Perilla, Department of Chemistry and Biochemistry, University of Delaware, Newark, DE, USA, Tel.: (302) 831-4806, Email: jperilla@udel.edu

MATERIALS AND METHODS

CA cross-linked hexamer protein expression and purification

U-^{13}C,^{15}N-enriched cross-linked hexamers were prepared as reported previously (1) with modifications. The gene was amplified and ligated into the pET21 vector using NdeI and XhoI sites, and *E. coli* Rosetta (DE3) cells were transformed with the vector. 5 mL of LB medium was inoculated with a single colony and cells were grown at 37 °C for 8-10 h to an OD of 1.5. Following centrifugation at 4000 g for 5 min at 4 °C, cells were resuspended in 50 mL of fresh modified M9 medium and grown overnight at 37 °C. This seeding culture was transferred into 1 L of modified M9 medium, containing 2 g/L $^{15}NH_4Cl$ and 2 g/L U-$^{13}C_6$-glucose. Cells were grown to 1.0-1.2 OD, induced with 0.8 mM IPTG for protein expression at 18 °C for 16 h. Cells were harvested by a centrifugation at 4000 g for 1 h at 4 °C, resuspended in a 25 mM sodium phosphate buffer (pH 7.0) and then stored at -80 °C. Cells were thawed and sonicated for 20 min (10 s on and 10 s off) in an ice bath. The lysed cells were centrifuged at 27,000 g for 1 h at 4 °C. The pH of the supernatant was adjusted to 5.8 with acetic acid, and the conductivity was reduced to below 2.5 ms/cm with de-ionized water. Following another centrifugation at 27,000 g for 1 h at 4 °C, the final supernatant was loaded onto a cation exchange column (HiTrap SP HP, 5 mL) and eluted with a 0-1 M NaCl gradient in buffer containing 25 mM sodium phosphate (pH 5.8), 1 mM DTT, 0.02% NaN_3. Concentrated protein fractions were further purified using a size-exclusion column (HiLoad 26/600 Superdex 75), equilibrated with a buffer containing 25 mM sodium phosphate (pH 7.0), 1 mM DTT, 0.02% NaN_3. The typical yield for U-^{13}C,^{15}N-enriched CA protein (monomer) is 10-15 mg/L.

Purified CA monomer proteins were first dialyzed into 25 mM sodium phosphate buffer (pH 7.5) without DTT or other redox reagents 48 h to achieve complete cross-linking. Then the clean cross-linked hexamers (~150 kDa) were separated from other oligomers by a size-exclusion column (HiLoad 16/600 Superdex 200). The fractions containing CA cross-linked hexamer were combined and concentrated to 20-30 mg/mL in 25 mM phosphate buffer (pH 8.0) by Amicon ultracentrifugal filter unit (Millipore, MWCO 10 kDa). The typical yield of concentrated protein is 8-10 mg/L after the Superdex 200 column.

U-^{13}C,^{15}N-labeled CA cross-linked hexamers were assembled into tubes from 26 mg/mL protein solution in 2.4 M NaCl, 25 mM phosphate solution (pH 7.5), followed by incubation at 37°C for 1 hour and overnight at 4°C. 53 mg of the tubular assemblies of cross-linked CA hexamers were packed into a Bruker thin wall 3.2 mm rotor by centrifugation.

CA A204C protein expression and purification

Natural abundance and U-^{13}C,^{15}N-enriched CA A204C proteins were expressed and purified as reported previously (2) with modifications. The gene was amplified and ligated into pET21 vector using NdeI and XhoI sites, and *E. coli* Rosetta (DE3) cells were transformed with the vector. To express natural abundance CA A204C protein, cells were pre-cultured in 25 mL Luria-Bertani (LB) medium (25 g/L) and grown at 37 °C overnight. Cells were transferred into 1 L of LB medium and grown at 37 °C till an OD of 0.7 for induction with 0.5 mM IPTG and protein was expressed at 25 °C for 16-18 h. For U-^{13}C,^{15}N-labeled CA A204C protein, cells were cultured in 2 L of Luria-Bertani (LB) medium and grown at 37 °C until till 1.2-1.4 OD. Following centrifugation at 4000 g for 30 min at 4 °C, cell pellets were washed with M9 medium, containing no ^{15}N and ^{13}C carbon sources and resuspended in 1 L of M9 medium, containing 2 g/L $^{15}NH_4Cl$, 2 g/L U-$^{13}C_6$-glucose. Cells were grown at 25 °C to an OD of 1.0-1.2, induced with 0.8 mM IPTG and protein was expressed at 18 °C for 18 h. Cells were harvested by centrifugation at 4000 g for 30 min at 4 °C, resuspended in 25 mM sodium phosphate buffer (pH 7.0), and sonicated for 20 min (10 s on and 10 s off) in an ice bath. Cell debris was removed by centrifugation at 27,000 g for 1 h at 4 °C. The pH of the supernatant was adjusted to 5.8 with acetic acid, and the conductivity was reduced to below 2.5 ms/cm with de-ionized water. Following another centrifugation at 27,000 g for 1 h at 4 °C, the supernatant was loaded onto a cation exchange column (HiTrap SP HP, 5 mL) and eluted with a 0-1 M NaCl gradient in buffer containing 25 mM sodium phosphate (pH 5.8), 1 mM DTT, 0.02% NaN_3. Concentrated protein fractions were further purified using a size-exclusion column (HiLoad 26/600 Superdex 75) equilibrated with buffer containing 25 mM sodium phosphate (pH 7.0), 1 mM DTT, 0.02% NaN_3. The fractions that contained CA A204C were combined and concentrated to 20 mg/mL in 25 mM phosphate buffer, pH 5.5, in an Amicon ultracentrifugal filter unit (Millipore, MWCO 10 kDa). During the concentration step CA A204C some protein precipitates and was removed by centrifugation. The typical yield for natural abundance protein is 6-10 mg/L and 5-6 mg/L for U-^{13}C,^{15}N-enriched protein. Final yield are 4-6 mg/L and 3 mg/L, respectively.

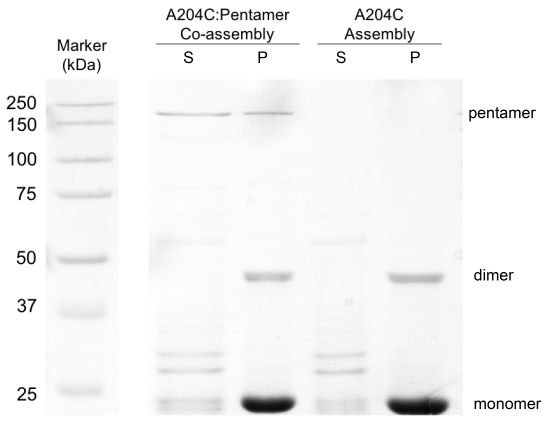
CA cross-linked pentamer protein expression and purification

U-^{13}C,^{15}N-enriched CA pentamers (N21C/A22C/W184A/M185A) were prepared as reported previously (3) with modifications. The CA N21C/A22C/W184A/M185A protein expression and purification followed the protocol for the expression and purification of U-^{13}C,^{15}N-enriched cross-linked hexamers described above. The typical yield for U-^{13}C,^{15}N-enriched CA N21C/A22C/W184A/M185A protein (monomer) is 10-15 mg/L.

To prepare clean cross-linked pentamers, purified CA monomer protein was first dialyzed into 25 mM sodium phosphate buffer, pH 8.0, without DTT or other redox reagents for 48 h to achieve complete cross-linking. Clean cross-linked pentamers (~125 kDa) were separated from other oligomers by size-exclusion chromatography (HiLoad 16/600 Superdex 200). The fractions that contain cross-linked CA pentamer were combined and concentrated to 20 mg/mL in 25 mM phosphate buffer, pH 8.0, in an Amicon ultra centrifugal filter unit (Millipore, MWCO 10 kDa). Since the efficiency of pentamer cross-linking is low, the typical yield is 2-3 mg/L after gel filtration.

Preparation of CA A204C cone-like assembly and CA A204C/pentamer conical co-assembly

U-^{13}C,^{15}N-labeled CA A204C was assembled into cone-like morphology from 20 mg/mL protein solutions in 25 mM phosphate buffer, pH 5.5, containing 2.4 M NaCl, followed by incubation at 37°C for one hour and 4°C overnight. The CA A204C assemblies were pelleted at 10,000 g, and 32 mg of the CA cone-like assemblies were packed into a Bruker thick wall 3.2 mm rotor. To prepare CA A204C/pentamer co-assemblies, 20 mg/mL CA cross-linked pentamers (U-^{13}C,^{15}N-labeled) were pre-assembled in 25 mM phosphate buffer, pH 8.0, containing 2.4 M NaCl, followed by incubation at 37°C for one hour. Natural abundance CA A204C protein solution was then added to the CA pentamer assemblies to yield a final molar ratio of 10:1 (A204C:pentamers). After incubating the mixed protein solution on a rotating platform at 25°C for 1h, the solution was adjusted to 25 mM phosphate buffer, pH 6.5, 2.4 M NaCl, and incubated overnight at 4°C. The CA A204C/pentamer cone-like co-assemblies were pelleted at 10,000 g, and 23 mg of these co-assemblies were packed into a Bruker thick wall 3.2 mm rotor. The efficiency of the A204C assembly and the incorporation of pentamers was verified by a cosedimentation assay, as shown below (S, supernatant; P, pellet):



Strep-TEV-Rhesus Trim5α (residue 133-497)-His6.

The cDNA encoding the CC-SPRY domain (residues 113-497) of Rhesus Trim5 alpha, containing Strep-tag followed by a TEV protease cleavage site at the N-terminus and a His_6-tag at the C-terminus was inserted into the pENT-TOPO vector (Life Technologies, Inc.). Baculoviruses expressing CC-SPRY were prepared using BaculoDirect C-term (Life Technololgies, Inc.), according to the manufacturer's protocol. Recombinant proteins were expressed in SF21 cells (Life Technologies, Inc.) for 40 h after infection with a multiplicity of infection of 2. Proteins were purified over a 5 mL Ni-NTA column (GE Lifesciences, Inc.) followed by a Superdex200 16/60 gel filtration column (GE Lifesciences, Inc.), equilibrated with 25 mM TrisHCl, pH 7.5, 150 mM NaCl, 1 mM DTT, 10 % glycerol, 0.02 % sodium azide.

The primary sequence is given below, with the residues of the tags shown in red:
MASWSHPQFEKGAENLYFQSEFGTATMEEVAQEYHVKLQTALEMLRQKQQEAEKLEADIREE
KASWKIQIDYDKTNVSADFEQLREILDWEESNELQNLEKEEEDILKSLTKSETEMVQQTQYMRE
LISELEHRLQGSMMDLLQGVDGIIKRIENMTLKKPKTFHKNQRRVFRAPDLKGMLDMFRELTDA
RRYWVDVTLATNNISHAVIAEDKRQVSSRNPQIMYQAPGTLFTFPSLTNFNYCTGVLGSQSITS
GKHYWEVDVSKKSAWILGVCAGFQSDAMYNIEQNENYQPKYGYWVIGLQEGVKYSVFQDGS
SHTPFAPFIVPLSVIICPDRVGVFVDYEACTVSFFNITNHGFLIYKFSQCSFSKPVFPYLNPRKCT
VPMTLCSPSSLEHHHHHH

Preparation of TRIM5α CC-SPRY-bound tubular assemblies of cross-linked CA hexamers

For studies of TRIM5α CC-SPRY binding, U-^{13}C,^{15}N-labeled CA cross-linked hexamers were first assembled into tubes from 26 mg/mL protein solution in 2.4 M NaCl, 25 mM phosphate solution (pH 5.5), followed by incubation at 37°C for 1 hour and overnight at 4°C. Before pelleting, an approximately equal volume of CC-SPRY storage buffer (25mM Tris, 150 mM NaCl, 0.02% NaN_3, 2 mM DTT, 10% glycerol) was added (giving a final NaCl concentration of 1M) and incubated on a rotating platform at 25°C for one hour with mixing at 15 minute intervals to replicate the sample preparation of the TRIM5α-bound tubes as described below. Using the pelleting procedure above, 19 mg of the hexameric CA tubes were packed into a Bruker thin wall 3.2 mm rotor. For the TRIM5α-bound sample, the CC-SPRY truncated construct (coiled-coil and PRY/SPRY domains) of Rhesus TRIM5α was used. TRIM5α-CA complex was formed as reported by Zhang and co-workers with a capsid-to-CC-SPRY ratio of 3:1 (47, 60). CA tubes were pre-assembled from 26 mg/mL protein solutions in 25 mM phosphate buffer (pH 5.5) containing 2.4 M NaCl, followed by incubation at 37°C for one hour and 4°C overnight. Rhesus TRIM5α CC-

SPRY (details on the construct and purification are given in Supporting Information) at a concentration of 3-4 mg/mL was added to the preassembled CA tubes, to 1.0 M final NaCl concentration and incubated for 1 hour on a rotating platform at room temperature with gentle mixing at 15 min intervals. The complex was then pelleted at 15,000 g, 4°C in a Beckmann ultracentrifuge. 15 milligrams of the complex was packed into a Bruker thin wall 3.2 mm rotor.

Solid-state NMR spectroscopy

One-dimensional CP and double cross polarization (DCP) buildup curves of tubular assemblies of cross-linked hexamers, A204C cone-like, and A204/pentamer cone-like co-assemblies were acquired at 14.1 T, with Larmor frequencies of 600.8 MHz (^{1}H), 150.8 MHz (^{13}C) and 60.8 MHz (^{15}N) with a Bruker 3.2 mm E-free HCN probe at temperatures of 4±1°C and 14±1°C. ^{13}C and ^{15}N chemical shifts were referenced with respect to the external standards adamantane and ammonium chloride, respectively. MAS NMR spectra were collected at a magic angle spinning frequency of 14.000 ± 0.002 kHz regulated by a Bruker MAS controller. Typical 90^{o} pulse lengths were 2.8 μs (^{1}H), 4.25 μs (^{13}C), and 4.05 μs (^{15}N) for the hexamers, 3.5 μs (^{1}H), 4.4 μs (^{13}C), and 4.05 μs (^{15}N) for the hexamer-pentamer co-assembly, 2.9 μs (^{1}H), 4.7 μs (^{13}C), and 4.9 μs (^{15}N) for A204C, and 2.8 μs (^{1}H), 4.25 μs (^{13}C), and 4.45 μs (^{15}N) for the A204C-pentamer co-assembly. ^{1}H-^{13}C and ^{1}H-^{15}N CP was achieved with a linear amplitude ramp of 90-110%, with the ^{1}H radio frequency (rf) field of 80-85 kHz matched to Hartmann-Hahn conditions at the first spinning sideband. Typical ^{1}H-^{13}C and ^{1}H-^{15}N contact times were 0.8-1.1 ms and 1.5-2 ms, respectively. Typical DCP power levels of 30-42 kHz on ^{13}C and 55-60 kHz on ^{15}N were used with optimized ^{15}N-^{13}C DCP contact time of 5-5.5 ms. SPINAL-64 decoupling (~100 kHz) was applied during the evolution and acquisition periods. The ^{1}H field strength during CORD was 12.5 kHz, and the CORD mixing time was 50 ms. The ^{13}C-^{13}C 2-dimensional CORD spectrum of A204C/U-^{13}C,^{15}N pentamer co-assemblies was acquired with non-uniform sampling (NUS (61)) with 40% sampling and a 2T_2 bias (144 complex points).

In the 2D RN-DIPSHIFT recoupling experiments (4), rotor synchronized $R10_1^3$ symmetry blocks using a pi pulse as the basic R element were incorporated during the t_1 period for ^{1}H-^{13}C dipolar evolution, for a total of 16 t_1 increments. The number of scans was 128 for hexamer tubes, 8192 for hexamer-pentamer co-assembly, 5120 for A204C, and 5120 for the A204C-pentamer co-assembly. The radio frequency field strength applied on the ^{1}H channel was 70 kHz in all experiments.

A sequence of ^{13}C{^{15}N} rotational echo double resonance (REDOR) experiments (5) were conducted with dephasing times ranging from 0.071 ms to 8.45 ms. The number of scans for

experiments with (S) and without REDOR pulses (S_0) were 440 for hexamer tubes, 3552 for hexamer-pentamer co-assembly, 3552 for A204C, and 11264 in the case of the A204C-pentamer co-assembly. The ^{15}N RF power applied during REDOR was 62kHz for hexamers, 57kHz for hexamer-pentamer co-assembly, 51kHz for A204C, and 56kHz for A204C-pentamer co-assembly. The XY-8 phasing scheme was applied during the rotor synchronized REDOR π pulse train. High-powered SPINAL64 ^{1}H decoupling at 75 kHz was used in all experiments. Error bars in the REDOR dephasing curve were calculated with the following equation:

$$\sigma = \left(\frac{S}{S_0}\right)\sqrt{\left(\frac{1}{SNR(S)^2} + \frac{1}{SNR(S_0)^2}\right)}$$

where SNR is the signal-to-noise ratio.

NMR data processing and analysis

All spectra were processed in TopSpin and with NMRpipe (62) and analyzed using SPARKY (63) and CCPNMR (64). For 2D data sets, 30°, 45°, or 60°-shifted sine bell apodization followed by a Lorentzian-to Gaussian transformation was applied in both dimensions. Forward linear prediction to twice the number of the original data points was used in the indirect dimension in some data sets, followed by zero filling to twice the total number of points. Spectra of Spectra were assigned by comparison with CA resonance assignments as reported previously (42). 1D REDOR spectra were processed without apodization. The Cα spectral range was integrated and all integrated intensities were normalized to the greatest intensity of the buildup curve.

Numerical simulations of DIPSHIFT and REDOR data

Numerical simulations of ^{1}H-^{13}C RN-DIPSHIFT lineshapes and ^{13}C{^{15}N} REDOR dephasing curves were performed in SIMPSON (6). To produce a powder average for the ^{1}H-^{13}C DIPSHIFT simulations, a total of 320 pairs of {α,β} angles were generated according to the REPULSION algorithm and sixteen γ angles. Best-fit parameters were obtained with the Minuit subroutine in SIMPSON version 1.1.2.

All REDOR dephasing curve simulations were performed for 5-spin systems, which included two $^{15}N^H$, $^{13}C^\alpha$, $^{13}C^\beta$, and ^{13}C'. Heteronuclear $^{15}N^H$-$^{13}C^\alpha$ as well as homonuclear $^{13}C^\alpha$-$^{13}C^\beta$ and $^{13}C^\alpha$-^{13}C' couplings were included. The rigid-limit N-C$^\alpha$ dipolar coupling constant is 984 Hz. For REDOR dephasing curve simulations of the tubular assemblies of cross-linked hexamer U-^{13}C,^{15}N-CA A14C/E45C/W184A/M185A and of the conical assemblies of U-^{13}C,^{15}N-CA A204C,

the simulations required two distinct effective N-C^{α} dipolar couplings of 750 Hz and 550 Hz. For REDOR dephasing curve simulations of the conical co-assemblies of 1:10 ratio of cross-linked pentamer U-^{13}C,^{15}N-CA N21C/A22C/W184A/M185A with natural abundance A204C, an N-C^{α} dipolar coupling of 850 Hz was used. Sample scripts and details of the spin systems are given below. Chemical shift anisotropies were not included in the simulations. All other parameters matched experimental values. A total of 320 pairs of $\{\alpha,\beta\}$ angles were generated according to the REPULSION algorithm and three γ angles were used to generate a dephasing curve.

SIMPSON script for REDOR dephasing curve simulation: pentamer/A204C conical coassembly

```
spinsys {
  channels 13C 15N
  nuclei   13C 15N 13C 13C
 #dipole 3 1 196.055 0 0 0
 dipole 1 2 850.000 0 0 0
 dipole 3 1 -2025.39 0 0 0
 dipole 4 1 -2025.39 0 0 0
# shift 2 0p 98p 0.2 0 0 0
# shift 3 0p 98p 0.2 0 0 0
# shift 5 0p 98p 0.2 0 0 0
}
par {
  proton_frequency 850e6
  spin_rate        14000
  sw               7000
  np               64
  crystal_file     rep320
  gamma_angles     3
  start_operator   I1x
  detect_operator  I1p
  verbose          1101
  variable rf      57000
  variable rfC     57000
}
proc pulseq {} {
  global par
  maxdt 1.0
  set t180 [expr 0.5e6/$par(rf)]
  set tr2 [expr 0.5e6/$par(spin_rate)-$t180]
  reset
  delay $tr2
  pulse $t180 0 x $par(rf) x
```

```
  delay $tr2
  pulse $t180 0 x $par(rf) y
  store 1
  reset
  acq
  delay $tr2
  pulse $t180 0 x $par(rf) x
  delay $tr2
  pulse $t180 $par(rfC) x 0 x
  prop 1
  store 2
  acq
  for {set i 2} {$i < $par(np)} {incr i} {
    reset
    prop 1
    prop 2
    prop 1
    store 2
    acq
  }
}
proc main {} {
  global par
  set    f [fsimpson]
  fsave $f $par(name).fid
}
```

SIMPSON script for REDOR dephasing curve simulation: cross-linked hexamer tubes and A204C cones

```
spinsys {
  channels 13C 15N
  nuclei   13C 15N 15N 13C 13C
 dipole 3 1 550.000 0 0 0
 dipole 1 2 750.000 0 0 0
 dipole 5 1 -2025.39 0 0 0
 dipole 4 1 -2025.39 0 0 0
# shift 2 0p 98p 0.2 0 0 0
# shift 3 0p 98p 0.2 0 0 0
# shift 5 0p 98p 0.2 0 0 0
}
par {
  proton_frequency 850e6
  spin_rate        14000
  sw               spin_rate/2.0
  np               64
  crystal_file     rep320
  gamma_angles     3
  start_operator   I1x
  detect_operator  I1p
  verbose          1101
  variable rf      62000
}
proc pulseq {} {
  global par
  maxdt 1.0
  set t180 [expr 0.5e6/$par(rf)]
  set tr2 [expr 0.5e6/$par(spin_rate)-$t180]
  reset
  delay $tr2
  pulse $t180 0 x $par(rf) x
  delay $tr2
```

```
  pulse $t180 0 x $par(rf) y
  store 1
  reset
  acq
  delay $tr2
  pulse $t180 0 x $par(rf) x
  delay $tr2
  pulse $t180 $par(rf) x 0 x
  prop 1
  store 2
  acq
  for {set i 2} {$i < $par(np)} {incr i} {
    reset
    prop 1
    prop 2
    prop 1
    store 2
    acq
  }
}
proc main {} {
  global par
  set    f [fsimpson]
  fsave $f $par(name).fid
}
```

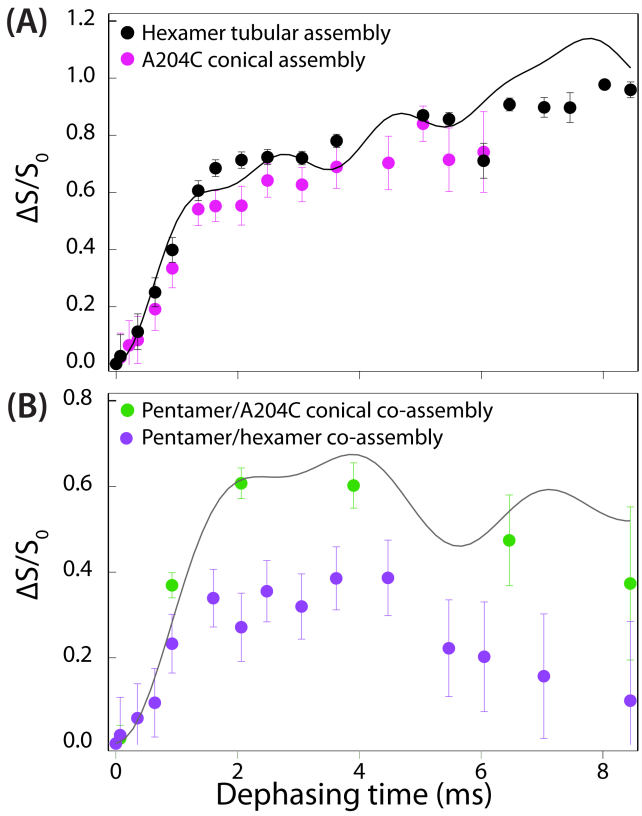


Figure S1. ^{15}N-^{13}C REDOR dephasing curves for (A) tubular assemblies of U-^{13}C,^{15}N cross-linked hexamers (black) and cone-like assemblies of CA A204C (magenta); and (B) cone-like co-assemblies of 10:1 natural abundance A204C/U-^{13}C,^{15}N cross-linked pentamers (light green) and co-assemblies of 10:1 natural abundance cross-linked hexamers/U-^{13}C,^{15}N cross-linked pentamers (purple). Fits are shown as black solid lines. Fits for hexamers in the context of tubular assemblies of cross-linked hexamers and cone-like assemblies of A204C require two environments, corresponding to effective ^{13}C-^{15}N dipolar coupling constants of 750 and 550 Hz, respectively. Fits for pentamers in the context of conical co-assembly with A204C require a single environment, corresponding to an effective ^{13}C-^{15}N dipolar coupling constant of 850 Hz. The rigid limit value is 984 Hz.

Dynamic Signatures of Hexameric and Pentameric Subunits in Capsid Assemblies: Cross Polarization and Double Cross Polarization

To further examine possible differences in dynamics, cross polarization (CP) and double cross polarization (DCP) buildup curves were recorded. Magnetization transfer time profiles in CP/DCP experiments are determined by the ^{1}H-^{13}C, ^{1}H-^{1}H and (for DCP) ^{15}N-^{13}C dipolar couplings, i.e. sensitive to motions on micro- to millisecond timescales (7). The C^{α} integrated intensities were used as a probe for dynamics. Remarkably, the ^{15}N-^{13}C DCP and the ^{1}H-^{13}C CP build-up curves (Fig S2, Tables S2 and S3) reveal that cross-linked pentamers in the co-assemblies with cross-linked hexamers are more rigid on the micro- to milliseconds timescale than the cross-linked hexamers in the tubular assemblies. Specifically, the effective rates of DCP decay are faster for the cross-linked pentamers in the conical co-assemblies ($\tau_{buildup}$ = 2.1 ms, τ_{decay} = 11.2 ms) than for the cross-linked hexamers in the tubular assemblies ($\tau_{buildup}$ = 2.2 ms, τ_{decay} = 24.8 ms) (rates were calculated assuming mono-exponential behavior). Similarly, the effective rates of the CP decay are faster for the cross-linked pentamers in the conical co-assemblies (τ_{decay} = 18 ms) than for the cross-linked hexamers in the tubular assemblies (τ_{decay} = 14 ms).

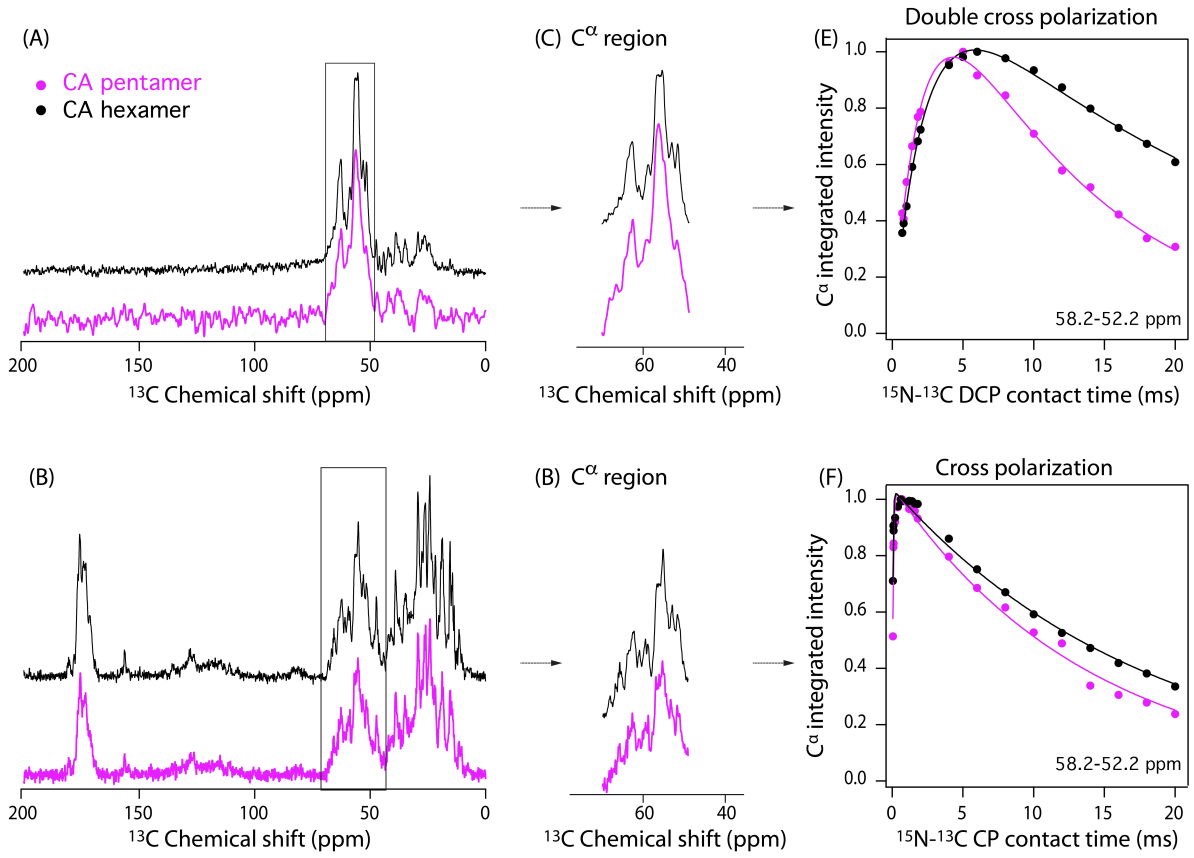
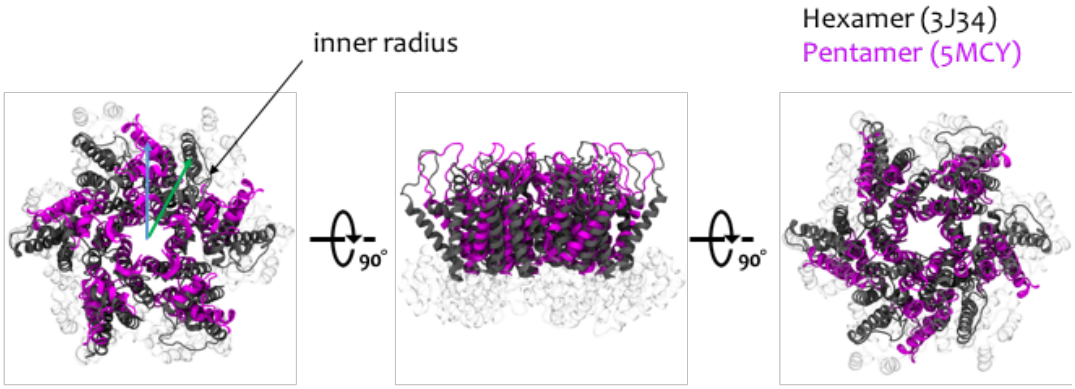


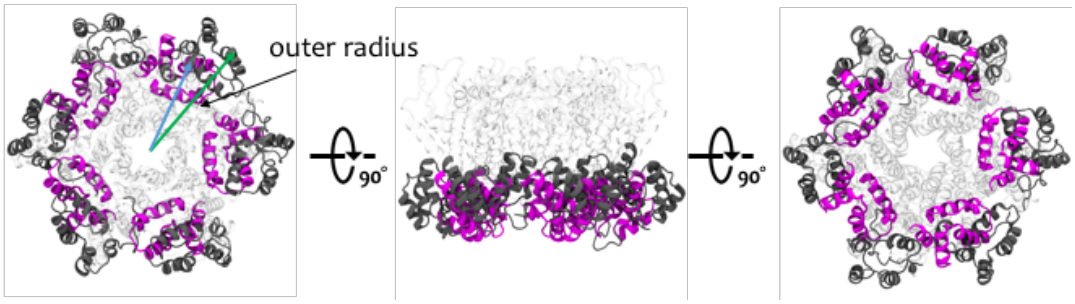
Figure S2. (B) ^{15}N-^{13}C double cross polarization (DCP, top) and ^{1}H-^{13}C cross polarization (CP, bottom) spectra (left panels) and build-up curves (right panel) for tubular assemblies of U-^{13}C,^{15}N cross-linked hexamers (black) and co-assemblies of natural abundance cross-linked hexamers with 10% U-^{13}C,^{15}N cross-linked pentamers (magenta).

NTD:



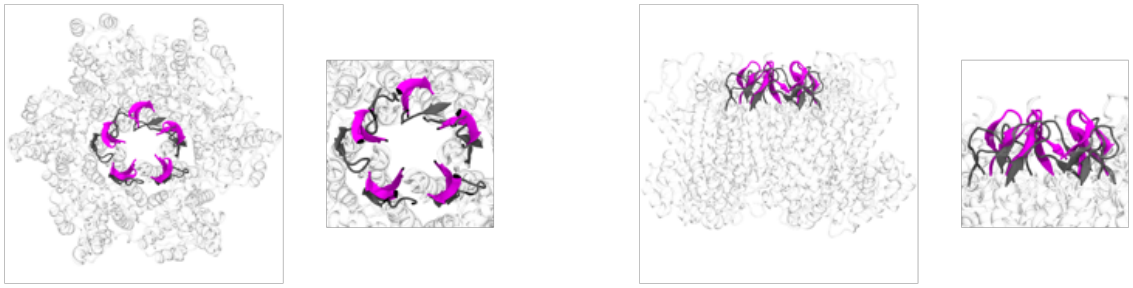
CTD:

In the pentamers, the CTDs are more closely packed, compared to the hexamer.

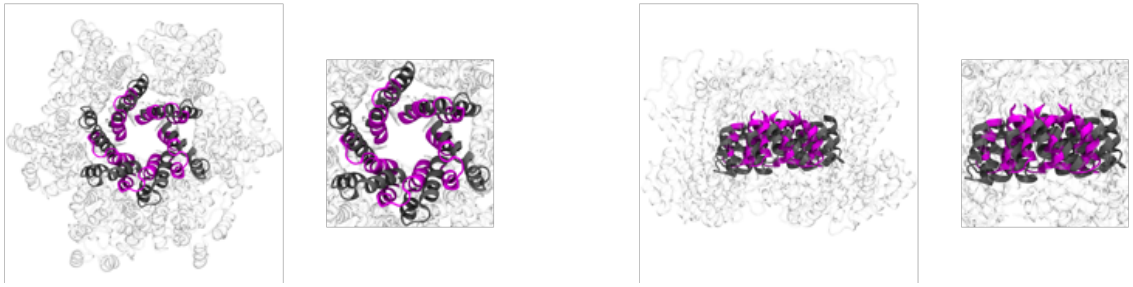


Beta-hairpin:

Compared to the six beta-hairpins in the hexamers, the five beta-hairpins in the pentamers are not as closely positioned.



Helices 1 and 2:



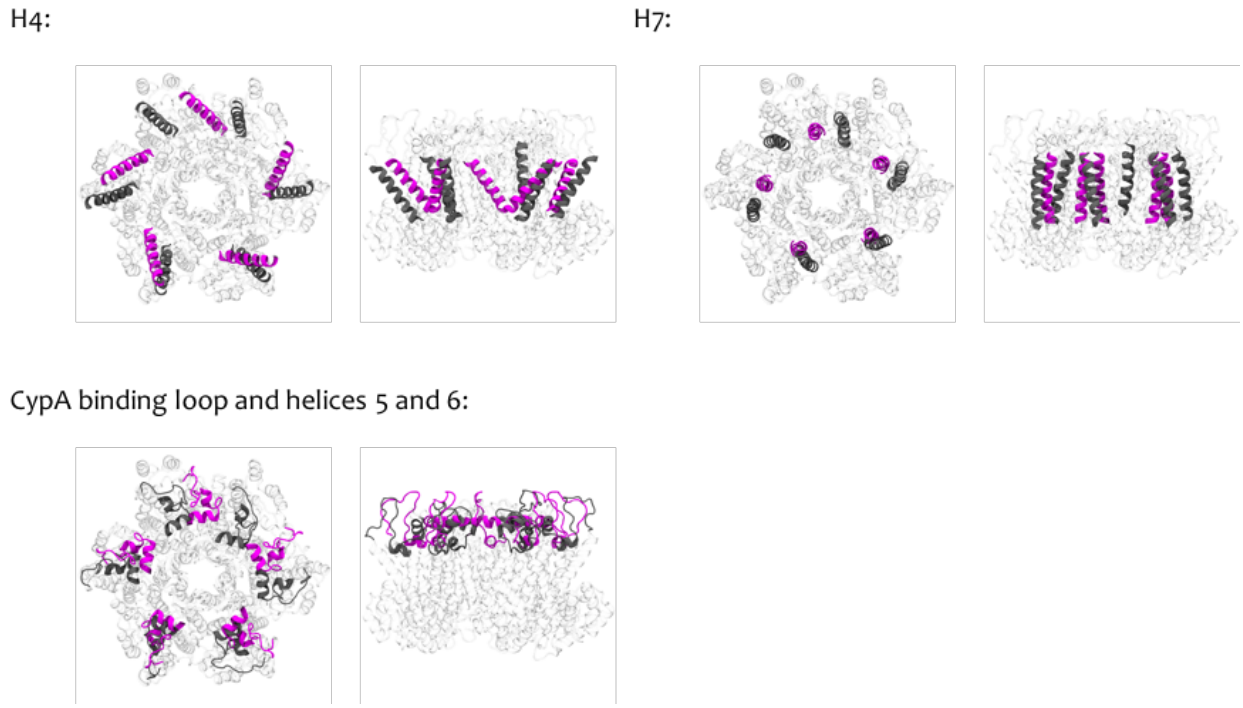


Figure S3. Summary of structural differences in the hexamers and pentamers.

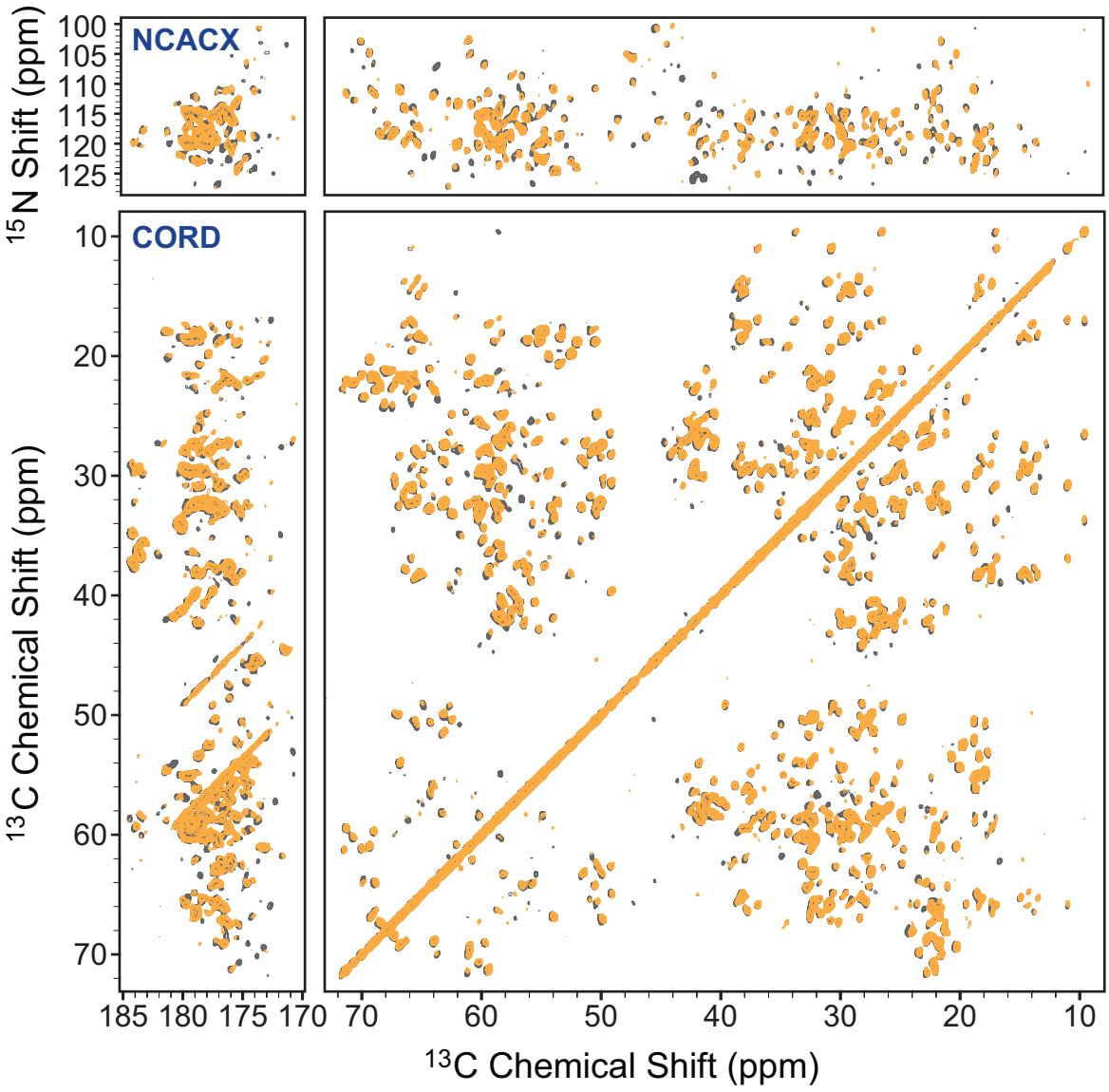


Figure S4. ^{13}C-^{13}C and ^{15}N-^{13}C correlation spectra of cross-linked CA A14C/E45C/W184A/M185A hexamer tubes in the absence (grey) and presence of TRIM5α CC-SPRY (orange).

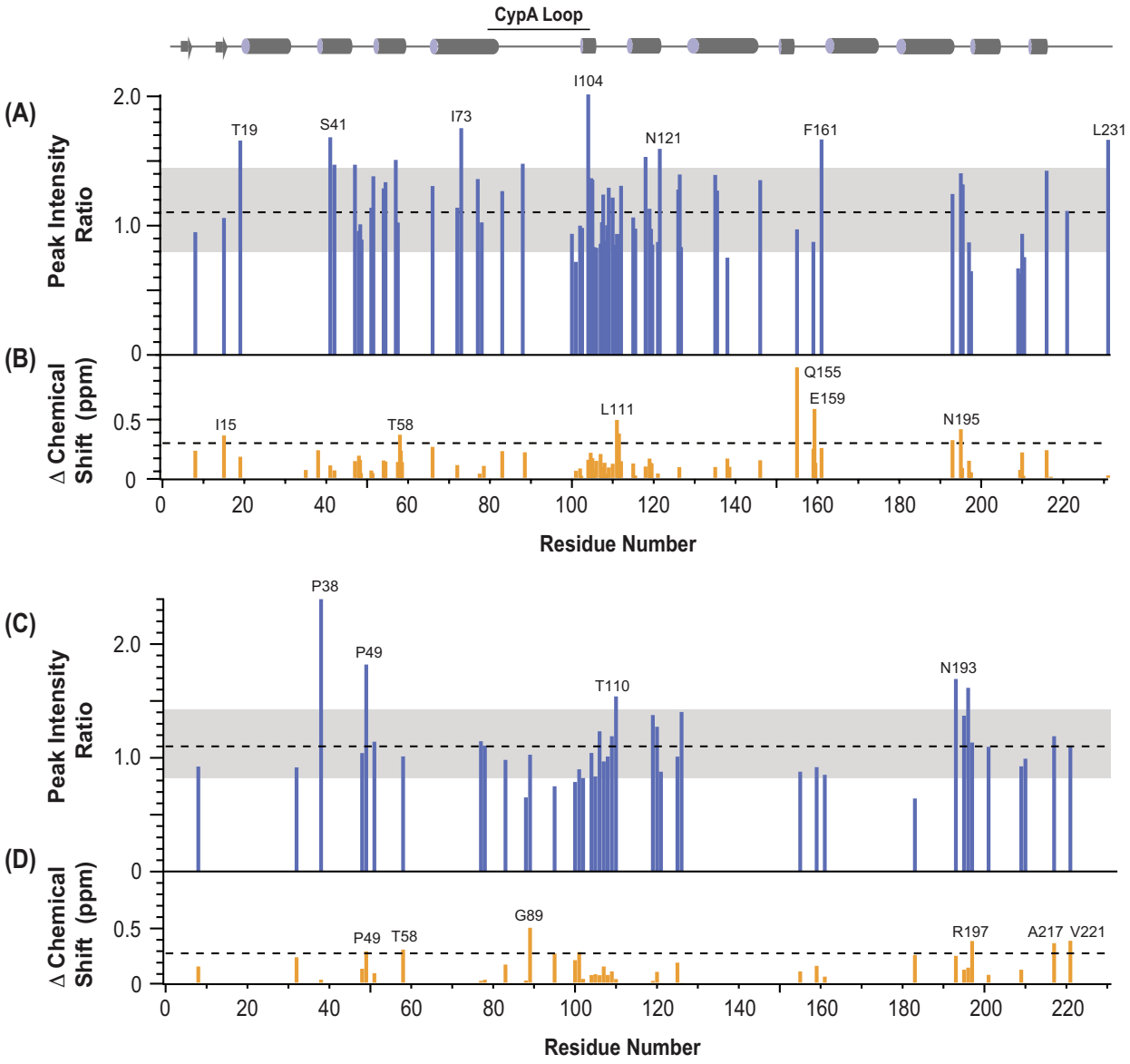


Figure S5. (A, C) Normalized peak intensity ratios in ^{15}N-^{13}C NCACX (A) and NCA (C) correlation spectra of TRIM5α CCSPRY-bound CA assemblies to free CA assemblies. Ratios greater than 1 standard deviation above or below the average (dashed line) are considered significant (outside the grey box). (B, D) Combined weighted ^{15}N,^{13}C chemical shift changes of CA resonances upon binding of TRIM5α CC-SPRY, extracted from the ^{15}N-^{13}C NCACX (B) and NCA (D) correlation spectra. The dashed line at ~0.3 ppm signifies the average RMSD. At the top, a schematic representation of the CA secondary structure is provided, with arrows indicating beta strands and cylinders alpha helices. The location of the CypA binding loop is also shown.

Table S1. Difference in linewidths for isolated resonances of TRIM5α CCSPRY-bound CA hexamer tubes vs free CA hexamer tubular assemblies, extracted from the direct ^{13}C-dimension in 2D ^{13}C-^{13}C CORD spectra, processed with a 30° sinebell. Negative values indicate a narrower linewidth in the CCSPRY-CA complex, while positive values indicate broader lines in the complex.

Residue	ω_1	ω_2	Δ linewidth
P1	62.3	24.8	-20.5
P1	24.7	62.4	-1.4
P1	50.3	62.4	5.2
P1	24.7	33.2	8.4
P1	24.7	50.3	8.7
P1	50.2	24.8	24
V3	21.3	35.7	10.3
V3	35.8	21.4	10.8
V11	21.8	36.4	14.3
I15	16.5	62.0	-68
I15	61.9	38.8	16.7
N21	38.6	55.8	-0.3
N21	55.8	38.7	31.8
A31	53.0	17.6	-6.8
P34	28.0	31.6	8.8
P34	50.8	31.6	14
P34	31.6	50.8	17.6
I37	18.2	13.5	-12.6
I37	13.4	18.2	-7.4
P38	30.5	28.1	-16.5
P38	48.9	28.1	-9.9
P38	64.7	49.1	0.8
P38	49.0	64.8	5.8
P38	28.0	49.1	8.5
P38	30.5	64.8	25
P38	28.1	30.7	36.7
P38	64.7	30.6	49.8
M39	32.6	15.9	4.7
F40	61.8	37.9	-18.3
S41	62.8	61.6	10.3
A42	54.2	20.0	2.8
A42	20.0	54.3	25.5
A47	53.4	20.6	19.8
T48	59.7	69.0	13.6
N57	54.7	37.4	13

N57	37.4	54.7	17.1
T58	63.6	23.4	-17.9
T58	23.3	69.8	-10.4
M68	17.9	58.1	-8
E75	59.2	36.2	2
E79	58.3	34.7	-13.2
E79	28.8	34.7	2.5
E79	34.6	28.9	8.9
R82	43.2	59.3	-13.7
R82	59.1	43.1	-13.6
R82	28.0	43.2	-9.7
R82	43.0	30.8	27.4
L83	42.7	55.1	-1.9
L83	55.1	22.3	13.5
P90	49.9	63.1	13.4
A92	50.7	17.8	16
E98	36.6	54.2	-5.4
E98	54.2	36.8	6.5
P99	26.3	49.2	-34.1
P99	62.6	26.3	-27.1
P99	62.6	30.3	-13.2
P99	49.1	62.6	-9.3
P99	62.5	49.1	23.7
P99	30.3	62.7	26.5
R100	52.8	30.6	-118.1
R100	30.5	53.0	-85.4
S102	60.7	62.0	-3.1
S102	62.0	60.7	20.9
I104	10.9	36.9	-19.2
I104	36.7	10.9	-11.9
I104	10.9	16.9	-9.8
I104	30.6	10.9	-1
I104	65.8	10.9	1
I104	10.9	10.9	2.3
I104	36.7	16.9	2.5
I104	16.8	36.8	4.3
I104	10.9	30.7	6.7
I104	16.9	11.0	7.8
I104	65.8	36.9	14
A105	50.4	18.7	3.8

A105	18.6	50.4	5.2
T107	63.9	68.9	6
T107	68.8	63.9	14.9
T108	60.8	69.3	-2.4
T108	60.9	20.3	3.4
T108	20.2	69.3	4.7
T108	69.2	20.2	5.4
T108	20.2	60.9	11.4
S109	53.9	66.7	-5.5
S109	66.7	54.0	-0.7
T110	59.3	71.2	-0.4
T110	71.2	59.4	8.2
L111	22.1	40.5	20
I115	17.4	29.0	-25.4
I115	38.4	17.3	-3.7
I115	28.9	17.3	-3.6
M118	35.0	33.2	10.6
M118	33.2	35.1	22.1
T119	61.0	21.6	11.5
T119	21.4	61.0	22
T119	61.0	70.0	27.1
N121	39.5	49.1	32.1
I124	14.5	27.6	-13.9
I124	27.6	14.6	-5.3
I124	17.2	59.4	8.3
I124	27.6	17.2	21.3
P125	51.2	27.2	5.1
P125	27.1	51.2	22.2
V126	30.7	23.5	-26.9
V126	19.3	65.7	-18.7
V126	23.5	65.7	-12.3
V126	19.4	23.6	-6.9
V126	30.8	19.4	0.5
V126	65.7	19.4	0.9
V126	65.7	23.5	4.1
V126	23.5	19.4	5.5
V126	19.3	30.8	6.4
V126	23.4	30.8	31.9
Y130	36.3	55.9	7.4
R132	28.1	44.1	12.8

I135	17.2	13.9	-28.4
I135	37.7	31.1	-14.6
I135	37.6	13.8	-14.4
I135	31.0	37.7	-13.2
I135	13.6	37.6	-11.5
I135	66.3	13.7	-8
I135	31.0	13.7	-2.3
I135	13.8	17.3	1.3
I135	13.6	31.0	1.7
I135	17.2	66.4	4.5
T148	71.4	61.1	-47.2
T148	61.1	22.7	2.3
T148	61.2	71.4	10.5
I153	16.9	26.5	-46.2
I153	16.9	33.8	-41.3
I153	9.4	26.5	-14.9
I153	26.5	9.5	-5.3
I153	9.5	17.0	-3.3
I153	33.7	17.0	-2.7
I153	26.5	17.0	-1.9
I153	9.4	9.5	5.5
I153	33.6	9.5	8.1
I153	9.5	33.7	11.2
I153	17.0	9.5	12.8
Q155	55.5	32.6	-9.1
K158	24.9	54.1	-5.5
E159	32.0	53.6	-40.2
E159	32.1	35.0	-36.9
E159	34.8	53.5	-27
E159	34.9	32.2	13.3
N193	52.6	38.3	21
P196	32.4	66.9	-16.5
P196	49.9	28.3	-4.6
P196	28.3	50.0	2
P196	66.8	28.3	14.7
P196	28.3	66.8	24.7
A209	19.6	52.5	5.1
A209	52.4	19.7	18.3
A217	56.0	18.7	-11.8
A217	18.6	56.0	-9.6

V221	64.2	20.9	5.7
V221	32.5	20.9	6.8
V221	20.9	64.3	13.3
L231	56.4	26.3	-59.9

Table S2. Summary of parameters in fits of ^{15}N-^{13}C double cross polarization (DCP) and cross polarization (CP) dynamics.

Sample	DCP		CP	
	T_{HH} (ms)	T_{CN} (ms)	T_{HH} (ms)	T_{CH} (ms)
U-^{13}C,^{15}N-cross-linked hexamer CA, tubular assembly	24.8 ± 0.8	2.2 ± 0.1	18.1 ±0.7	0.04 ±0.01
U-^{13}C,^{15}N-cross-linked pentamer CA, coassembly with n.a. cross-linked hexamer	11.2 ± 0.5	2.1 ± 0.1	14.0 ±0.6	0.06 ±0.01

The spectral region of 58.2-52.2 ppm, corresponding to Cα resonances, was integrated for the analysis of the DCP and CP dynamics.

The magnetization of double cross polarization vs. contact time curves were fit to the following equation:

$$M(t) = \frac{M_0}{\left(1 - \frac{T_{CN}}{T_{HH}}\right)} \times \left(e^{-\left(\frac{t}{T_{HH}}\right)} - e^{-\left(\frac{t}{T_{CN}}\right)} \right)$$

where M_0 is the initial ^1H-spin magnetization, T_{CN} is the effective time constant for ^{15}N-^{13}C magnetization build-up and T_{HH} is the effective time constant for the relaxation in the rotating frame.

The magnetization of cross polarization vs. contact time curves were fit to the following equation:

$$M(t) = \frac{M_0}{\left(1 - \frac{T_{CH}}{T_{HH}}\right)} \times \left(e^{-\left(\frac{t}{T_{HH}}\right)} - e^{-\left(\frac{t}{T_{CH}}\right)} \right)$$

where M_0 is the initial ^1H-spin magnetization, T_{CH} is the effective time constant for ^1H-^{13}C magnetization buildup, T_{HH} is effective time constant for the relaxation in the rotating frame.

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