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Dynamic conformational changes in the rhesus TRIM5α dimer dictate the potency of HIV-1 restriction

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\textbf{A B S T R A C T}

The TRIM5α protein from rhesus macaques (rhTRIM5α) mediates a potent inhibition of HIV-1 infection via a mechanism that involves the abortive disassembly of the viral core. We have demonstrated that alpha-helical elements within the Linker 2 (L2) region, which lies between the SPRY domain and the Coiled-Coil domain, influence the potency of restriction. Here, we utilize single-molecule FRET analysis to reveal that the L2 region of the TRIM5α dimer undergoes dynamic conformational changes, which results in the displacement of L2 regions by 25 angstroms relative to each other. These data suggest a model in which conformational changes in the L2 region mediate displacement of CA bound SPRY domains to induce the destabilization of assembled capsid during restriction.

1. Introduction

TRIM5α is a retroviral restriction factor which mediates a post-entry block to infection (Sastri and Campbell, 2011; Stremlau et al., 2004). The most well studied example of this restriction is the ability of the TRIM5α protein from rhesus macaques (rhTRIM5α) to potently inhibit HIV-1 infection (Sastri and Campbell, 2011; Stremlau et al., 2004). Like other members of the TRIM family of proteins, TRIM5α possesses the canonical RING, BBox2, and coiled coil (CC) domains that comprise the TRIPartite Motif that defines this family of proteins (Ozato et al., 2008). Like other TRIM family proteins, TRIM5α exhibits a strong tendency to self-associate into macromolecular assemblies in cells (Cai et al., 2008; Campbell et al., 2007). The N-terminal RING domain of TRIM5α is known to act as an E3 ubiquitin ligase (Pertel et al., 2011; Tareen and Emerman, 2011; Yamanauchi et al., 2008; Yudina et al., 2015), and, together with the BBox2 domain, also functions to mediate the self-association of TRIM5α dimers (Diaz-Griffero et al., 2009; Li et al., 2011). The CC domain, in cooperation with the Linker 2 (L2) region, mediates the dimerization of TRIM5α monomers and the formation of higher order assemblies (Goldstone et al., 2014; Kar et al., 2011; Langelier et al., 2008; Sanchez et al., 2014; Sastri et al., 2010). TRIM5α also possesses a C-terminal SPRY domain, which is known to recognize determinants in the assembled viral core to mediate restriction (Ohkura et al., 2006; Stremlau et al., 2005; Yap et al., 2005). Following core binding, TRIM5α induces the abortive disassembly of the viral core (Stremlau et al., 2006; Zhao et al., 2011), although the mechanism by which this abortive disassembly is induced by TRIM5α remains poorly understood.

Structural studies have been valuable in understanding the molecular basis for the interactions between TRIM5α and the HIV-1 capsid (CA) core. Cryo-EM studies have revealed that TRIM5α can form hexagonal assemblies on artificially assembled CA lattices (Ganser-Pornillos et al., 2011; Li et al., 2016). The domain organization of TRIM5α within this assembly has recently been revealed by structural studies revealing that TRIM5α and TRIM25, a closely related TRIM family member, form relatively long antiparallel dimers (Goldstone et al., 2014; Sanchez et al., 2014) (Fig. 1B), the dimensions of which are consistent with these dimeric units spanning each face of the hexagonal lattice observed by cryo-EM (Ganser-Pornillos et al., 2011) (Fig. 1C). As such, the antiparallel dimer consisting of the CC-L2-SPRY domain are thought to represent the basic CA binding unit of TRIM5α, and recombinant proteins comprised of the CC-L2-SPRY domains have been observed to bind assembled CA in vitro (Zhao et al., 2011). Moreover, this minimal CA binding unit, lacking the N-terminal RING...
and BBox2 domains, exhibited the ability to disrupt CA tubes in vitro, suggesting that the minimal components of TRIM5α which induce the abortive disassembly of the viral core are located in the CC-L2-SPRY fragment of TRIM5α (Zhao et al., 2011). Although the mechanism underlying the disruption of assembled CA by the CC-L2-SPRY fragment was not determined, in the absence of enzymatic activity, one possibility is that dynamic changes in the conformation of these domains cooperatively induce CA disassembly. Consistent with this hypothesis, the recently published structure of the TRIM5α dimer failed to resolve a stretch of residues in the L2 region (Goldstone et al., 2014) which our studies have found to regulate the ability of rhTRIM5α to restrict HIV-1 infection (Sastri et al., 2014, 2010). To test the hypothesis that this region undergoes dynamic conformational changes, we performed single-molecule Förster Resonance Energy Transfer (smFRET) experiments to monitor conformational changes in the CC-L2 dimer. Because the resonance energy transfer between donor and acceptor fluorophores is governed by the interfluorophore distance, smFRET is a powerful method to precisely measure conformational changes which occur in a protein. We observe that the WT rhTRIM5α dimer exhibits substantial conformational variability, exchanging among at least three conformations. Moreover, mutants which exhibited altered restriction exhibited altered occupancy of these FRET states as well as altered ability to transition between the states. Collectively, these results reveal that the rhTRIM5α dimer undergoes dynamic conformational changes and suggest a model where transitions between individual conformations might account for the ability of TRIM5α to induce the disassembly of CA assemblies.

2. Materials and methods

2.1. Recombinant DNA

To generate 6xHis-tagged CCL2 peptides, the CCL2 fragments WT rhTRIM5α and its L2 mutants (residues 132–296 of the full length protein) were cloned into the pET-15b vector by using the NdeI and BamHI restriction sites. To introduce a C-terminal cysteine on the CCL2 peptides, primers were generated against the C-terminal end of the CCL2 gene fragment containing the codon for cysteine and mutagenesis was performed by PCR mutagenesis.

2.2. Protein expression and purification

Transformed BL21(DE3) cells were grown in 0.25 l of Luria broth containing 100 μg/ml carbenicillin (Invitrogen) until the optical density at 600 nm (OD600) reached 0.6. The bacterial cultures were then induced to express WT or L2 mutant rhTRIM5α CCL2 peptides by adding 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) (Invitrogen) and shaking the cultures for 4 h at 37 °C. To purify 6xHis-tagged CCL2 peptides, bacterial pellets were lysed in a solution containing 50 mM Na2HPO4, 500 mM NaCl, 10 mM imidazole, 1% Triton X-100, 0.5 mg/ml lysozyme (Sigma), 8 M Urea, and a protease inhibitor cocktail (PIC) (Roche), followed by sonication. The lysates were then centrifuged at 13,000 rpm at 4 °C for 30 min. The pellet was discarded, and the supernatant was incubated with Talon metal affinity resin (Clontech) at 4 °C for 1–2 h with gentle mixing to facilitate binding of the His-tagged proteins to the resin. The mixture was passed through a 2-ml Talon disposable gravity column (Clontech) twice. The flow-through was discarded, and the resin was washed with a buffer...
containing 50 mM Na₂HPO₄, 500 mM NaCl, and 8 M urea (pH 8.0). The 6xHis-tagged proteins were eluted from the resin by using an elution buffer (50 mM Na₂HPO₄, 500 mM NaCl, 8 M urea, 300 mM imidazole). The protein fractions were analyzed by Coomassie staining of SDS-PAGE gels, and the fractions with the highest purity were dialyzed at 4 °C in decreasing concentrations of Urea (4 M, 2 M, and 0 M) for 3 h or overnight per step using 10,000 MWCO Slid-A-Lyzer Dialysis Cassettes (Thermo). The proteins were then spun at 10,000 rpm at 4 °C for 30 min to remove aggregates and final protein concentrations were determined by measuring the absorbance at 280 nm.

2.3. Protein labelling

Purified proteins were incubated with TCEP at 10x the protein concentration at 4 °C for 2 h or overnight to disrupt disulfide bonds. The proteins were then combined with a 3-fold excess of maleimide dye (Alexa Fluor 594 C5-maleimide or Alexa Fluor 488 C5-maleimide (Life Technologies)) in DMSO and incubated at 25 °C for 45 min. The reaction was incubated with 1 µM βME at 25 °C for 5 min to halt the labelling reaction. To separate free dye from protein, the sample was passed through a NAP-10 column (GE Healthcare) equilibrated with 50 mM sodium phosphate, pH 7, 1 mM DTT, 1.7 M (NH₄)₂SO₄ buffer. Proteins labelled with A488 or A594 fluorophores were denatured in 8 M urea, mixed at a 1:1 ratio and urea was removed by serial dialysis to 4 M, 2–0 M urea in 50 mM Na-phosphate, pH 7.4, at 4 °C in the dark.

2.4. Glutaraldehyde cross-linking assay

Glutaraldehyde cross-linking assays were performed as previously described (Sastri et al., 2014). Briefly, labelled purified proteins were incubated with 0, 1, 2, and 4 mM glutaraldehyde for 5 min at room temperature. The glutaraldehyde was saturated by the addition of 1 M glycine. The cross-linked proteins were then subjected to SDS-PAGE using 4%-to-15% gradient Tris-HCl gels (Ready Gels; Bio-Rad) and transferred to the nitrocellulose membrane. The cross-linked proteins were then subjected to SDS-PAGE in DMSO and incubated at 25 °C for 45 min. The proteins were then spun at 10,000 rpm at 4 °C for 30 min to remove aggregates and final protein concentrations were determined by measuring the absorbance at 280 nm.

2.6. Single-molecule FRET data analysis

Individual donor and acceptor intensity versus time traces were processed with custom Matlab software. Trajectories were corrected for 5% leakage of AlexaFluor 488 emission signal into the acceptor channel (determined previously) and background signal (determined from the average signal after photobleaching). Traces exhibiting single-step photobleaching and anti-correlated donor/acceptor signals were selected for further analysis. The apparent FRET efficiency, E, was calculated as E=[I_d]/[I_d+I_a], where I_d and I_a are the corrected donor and acceptor emission intensity, respectively. Composite histograms of FRET efficiency were compiled from multiple traces and fit with up to three Gaussian distributions using IGOR Pro software (Version 6, WaveMetrics). Individual FRET traces were fit to a three-state Hidden Markov Model using the software HaMMy ( McKinney et al., 2006). Transition density plots (TDP) (McKinney et al., 2006) were generated using custom Matlab scripts.

2.7. Molecular dynamics simulations

MD simulations were performed using GROMACS (Hess et al., 2008; Pronk et al., 2013) with the CHARMM 27 force field (Foloppe and MacKerell, 2000; MacKerell et al., 2004a, 2004b) and TIPS3P water model (Jorgensen et al., 1983). We used a previously described model of the rhTRIM5a CCL2 dimer as a starting structure (Sastri et al., 2014) and truncated this model at residue 296 to model the recombinant proteins used in this study. The starting system was minimized using the steepest descent method for 1000 steps, and then was solvated in a rectangular water box of with a minimum of 20 Å from the surface of the protein to the edge of the solvent box. Na⁺ and Cl⁻ ions were added to the solution to neutralize the charge of the system and to produce an ion concentration of 150 mM. The Particle Mesh Ewald (PME) method (Darden et al., 1993; Essmann et al., 1995) was used to describe long-range electrostatic interactions. Molecular dynamics simulations were carried out with an integration time step of 2 fs. To reach the target temperature (300 K) and pressure (1 bar), the Berendsen method (Berendsen et al., 1984) was used with relaxation time of 0.1 ps. After a 100 ps equilibration, after that we created two additional systems with harmonic restraints to keep Ca atoms of C-terminal cysteines at specific distances which were observed by smFRET (51 Å, 68 Å), and allowed the secondary structural elements of the L2 region to relax during a 10 ns molecular dynamics simulation. Production simulations were performed in the NPT ensemble using the Nose-Hoover thermostat (Hoover, 1985; Nose and Klein, 1983) and a Parrinello-Rahman barostat (Nose, 1984; Parrinello and Rahman, 1981) with relaxation times of 1.0 ps.

3. Results

3.1. Determinants of restriction and assembly in the rhTRIM5a dimer

Our previous studies have examined the role that residues within the L2 region play in viral restriction (23). These studies collectively revealed two classes of mutations. Restriction-abrogating mutations, such as the RRY275S-277AAA mutation, abrogate the ability of rhTRIM5 to inhibit HIV-1 infection (Fig. 2A and B). Conversely, restriction-enhancing mutations, such as the HKN271-273AAA mutation, exhibit inhibition of HIV-1 that is more potent than wt rhTRIM5 (Figs. 2A and B) (Sastri et al., 2014). Critical, circular dichroism analysis of the CC-L2 dimer revealed that the ability to inhibit HIV-1 correlated with the α-helical content of the dimer, such that mutations which abrogated HIV-1 restriction also exhibited reduced α-helical structure, while other mutations in the L2 region which increased the ability of rhTRIM5a to restrict HIV-1 exhibited more α-helical content than WT rhTRIM5a (Fig. 2B) (Sastri et al., 2014). However, mutations which abrogated restriction by disrupting α-helices in the L2 region...
were still able to bind assembled CA tubes in vitro (Fig. 2B) (Sastri et al., 2014). Collectively, these results suggest that secondary structural elements in the L2 region contribute to the poorly understood effector function during restriction which drives the abortive disassembly of the viral core.

To understand how residues of the L2 region interact with residues of the CC domain in the context of the CC-L2 dimer, we generated a homology model of the rhTRIM5α CC-L2 dimer in which the rhTRIM5α sequence was threaded into the TRIM25 structure and the structure was allowed to relax during a 10 ns molecular dynamics simulation (Fig. 2C). For consistency, we have maintained the structural designations developed by Sanchez et al. (2014) to describe the three α-helices present in the TRIM25 dimer. The first helix, H1, spans the entire CC domain and a short stretch of residues of the L2 region. The second helix, H2, is a short helix which forms a hairpin structure with residues in H1. The last helix, H3, is located in the center of the dimer and is docked to H1 of the alternate monomer (H1′) (Fig. 2C). This homology model revealed putative interactions between the 275RRV277 motif present on H3 and an acidic 177DYD179 motif present on H1’ (and H1 and H3’). Given that disrupting this interaction through mutation of the RRV motif substantially reduced the helical content of the CC-L2 dimer (Fig. 2B) (Sastri et al., 2014), this also suggested to us that this interaction between RRV 275–277 and DYD177–179 promotes the formation of H3, and similarly predicted that the L2 region might alternate between helical, docked and unstructured conformations. To directly test this hypothesis, we introduced C-terminal cysteines into the CC-L2 dimer (Residues 132–296 in the native protein) to allow fluorescent labelling of these cysteines using maleimide chemistry (Fig. 2D). There are no cysteines in the native rhTRIM5α CC-L2 sequence, ensuring specific labelling of the cysteines at this location in the CC-L2 peptide. Purified recombinant protein containing an N-terminal His tag was labelled with either A488 or A594 fluorescent probes. The two pools of labelled protein were then denatured, mixed in a 1:1 ratio and allowed to renature to generate CC-L2 dimers containing both A488 and A594 fluorescent probes. Glutaraldehyde crosslinking revealed that the introduction of cysteines and subsequent fluorescent labelling did not disrupt the ability of the CC-L2 peptide to form dimers, which were the predominant species observed following crosslinking (Fig. 2E).

3.2. smFRET reveals dynamic conformational changes in the rhTRIM5α dimer

We next performed Total Internal Reflection Fluorescent (TIRF) microscopy on fluorescently labelled CC-L2 dimers to monitor dynamic conformational changes that might occur in these dimers. Following acquisition, we focused our analysis on individual, dually labelled dimers by analyzing traces that 1) exhibited single step photobleaching of the donor fluorophore during the acquisition period and 2) fluctuations in donor and acceptor fluorescence were anti-correlated. In this way, any dimers not labelled with both fluorophores, and protein aggregates or accumulations representing more than single, dually labelled dimers, were removed from the analysis. FRET traces from individual WT-CC-L2 dimers revealed fluctuations among three distinct FRET states indicating that, under these conditions, the CC-L2 dimer exchanges between three conformations (Fig. 3A). Composite FRET histograms were fitted to three Gaussian distributions centered at apparent FRET efficiencies of 0.2, 0.6, and 0.8 (Fig. 3B). These apparent FRET efficiencies correspond to interfluorophore distances of approximately 68 Å, 51 Å and 43 Å, respectively. This observation would not be expected if distal elements of the L2 region became transiently undocked from the CC helix, as we originally hypothesized. Rather, these data suggest that, on the time scale of these experiments, the L2 arms of the dimer remain in relatively close proximity while sampling three distinct conformations. The fractional populations of the fitted Gaussian distributions reveal that the lowest FRET state was
sample most frequently (42%), while the high-FRET state was occupied the least (24%) and the middle FRET state was occupied 34% of the time prior to photobleaching. These data reveal that the WT CC-L2 dimer undergoes spontaneous transitions between three distinct conformations.

Idealized FRET trajectories were generated by fitting individual FRET traces data to a three-state Hidden Markov Model (Fig. 3C). The fitted FRET efficiencies for each state were in good agreement with the mean FRET efficiencies determined from the cumulative histograms. The idealized trajectories were used to generate a transition density plot, revealing the connectivity of the individual FRET states (Fig. 3D, Table 1). The TDP reveals that transitions occur among all three conformations, with transitions between the 0.2 and 0.6 FRET states being the most common and transitions between the 0.8 and 0.6 FRET states being the least common. When the 0.6 FRET state was occupied, the protein was ~5 times more likely to transition to the 0.2 FRET state (279 transitions observed) than to the 0.8 FRET state (50 transitions observed) (Table 1). Transitions between the most extreme FRET states, without detectable occupancy of the middle FRET state, were also observed, and in the case of transitions from the 0.8 FRET state, transitions to the 0.2 FRET state was ~4 times more frequent than to the intermediate FRET state (150 transitions vs 36 transitions) (Table 1).

We next examined the conformations of CC-L2 dimers harboring mutations in the L2 region which either abrogate (RRV275-277AAA) or enhance (HKN271-273AAA) HIV-1 restriction by full length rhTRIM5α. The HKN271-273AAA mutation, which enhances restriction of rhTRIM5α (Sastri et al., 2014), existed preferentially in the 0.6 (51 Å) FRET state (64%), and sampled the 0.2 (68 Å) and 0.8 (43 Å) FRET state less frequently (31% and 5% respectively) than WT (Fig. 4A). Moreover, the rate of transitions between conformations was noticeably slower in this mutant compared to WT, preventing transition frequency calculation using Markov Modelling. In contrast, the RRV275-277AAA dimer exhibited constant and low FRET efficiency over time, existing almost entirely around the 0.2 (68 Å) FRET state (Fig. 4B) observed in the WT dimer. Our original hypothesis had suggested that the RRV275-277AAA mutant would be unable to establish contacts between the L2 region and CC domains necessary for stable docking of the L2 region to Helix 1, preventing the observation of discrete FRET states. These data, however, suggest stable docking between L2 and CC regions in this mutant. Taken together with the smFRET traces obtained for the HKN271-273AAA mutant, these data suggest that the ability to assume the conformations associated with the 0.6 and 0.8 FRET states correlates to the ability to restrict HIV-1.

3.3. Molecular dynamics simulations of rhTRIM5α dimer conformations

We next used molecular dynamics simulations to establish models of the individual CC-L2 dimer conformations observed in our smFRET analysis. To generate these models, we used the TRIM25 homology model shown in Fig. 1A and introduced cysteine residues at the same position as our recombinant dimers (C297). To generate models for the observed FRET states, helices H3 and H3′ of this model were artificially separated by the observed separation distance by moving each cysteine an equivalent distance down the long axis of the dimer until the appropriate separation distance was achieved. H3 and H3′ were then artificially melted, after which the secondary structure in this region was allowed to reform in a 100 ns simulation (Fig. 5). In these models, the 68 Å separation model, which is the only conformation occupied by the RRV275-277AAA mutant, exhibited the least α-helical content in the residues which formed H3 in the 43 Å model. This is consistent with the observation that this mutant exhibits less α-helical content, as measured by circular dichroism (Fig. 2) (Sastri et al., 2014).

We also performed steered molecular dynamics (SMD) simulation in order to explore the mechanical/energetical properties of the transition between states, applying a spring constant of 600 kJ/mol/
resolve residues in the L2 region are precisely those residues that our previous studies have implicated as governing HIV-1 restriction (Sastri et al., 2014, 2010). Given the dynamic conformational changes observed using smFRET in this study, it is not surprising that this region of the protein was not amenable to x-ray crystallography. Therefore, our studies may explain, in part, the results of Goldstone et al., and conversely, the studies of Goldstone et al. (2014) provide insight into which residues in the L2 region are contributing to the conformational variability observed in our smFRET experiments.

These studies, taken together with our previous in vivo studies examining how mutations in the L2 region influence restriction and secondary structure (Sastri et al., 2014, 2010), provide insight into the individual conformations assumed by rhTRIM5α. In our previous studies, we have found that residues in the L2 region govern the ability of rhTRIM5α to restrict HIV-1 infection (Sastri et al., 2014, 2010), finding that the ability of the CC-L2 domain to adopt an extended α-helical structural transition was correlated to its ability to restrict infection. Restriction-defective mutations, such as RRV275-277AAA, reduced the helical content of the dimer, while restriction enhancing mutations, such as HKN271-273AAA, exhibited more α-helical structure than WT protein. Here, we observe that the WT rhTRIM5α dimer exhibits frequent conformational transitions, which correlate to displacements of the L2 termini 25 Å relative to each other. Given that the RRV275-277AAA mutant exclusively assumed the more extended (68 Å) conformation, and has less α-helical content than the WT dimer (Sastri et al., 2014), this suggests that the 68 Å conformation has less α-helical structure than the other two conformations observed in WT and the HKN271-273AAA mutant. By contrast, this suggests that the more compact conformations observed, in which the interferorophore distance was 51 Å or 43 Å, have more α-helical content than the extended conformation. This was observed in our molecular dynamics simulations performed to understand the structural basis of these conformations (Fig. 5).

Although this study focused exclusively on the basic dimeric unit of rhTRIM5α, it is worth considering the impact these conformational changes in the CC-L2 region would have on the full length dimer. In

4. Discussion

In this study, we performed smFRET measurements to demonstrate dynamic conformational changes occurring in the basic dimeric unit of rhTRIM5α, comprising the CC-L2 domain. Other studies have utilized similar constructs to obtain structural information of TRIM family dimers, notably TRIM25 (Sanchez et al., 2014) and TRIM5α (Goldstone et al., 2014). Notably, the structures described in these two studies differed substantially with respect to their ability to resolve the structure of the L2 region. The structure of TRIM25 reveals two well-ordered α-helices (H3, H3′) which dock along the long axis of the CC domains to generate a 4 helix bundle (Sanchez et al., 2014). By contrast, the structure of TRIM5α failed to resolve precise coordinates of these residues in the dimer, although an antiparallel dimer similar to the TRIM25 dimer was clearly resolved (Goldstone et al., 2014). Additionally, the residues in which the TRIM5 structure failed to resolve residues in the L2 region are precisely those residues that our
this context, the conformational transitions observed in the C-terminal portion of the L2 region are likely to affect the separation of the C-terminal SPRY domains which bind retroviral capsid assemblies. We speculate that this may be relevant to restriction via two distinct but not mutually exclusive mechanisms. First, variable spacing between SPRY domains may enhance the ability of TRIM5α proteins to bind a larger spectrum of CA assemblies with variably spaced binding sites. Second, SPRY translocation may drive the conserved ability of TRIM5α to disrupt the CA lattice during restriction (Stremlau et al., 2006; Zhao et al., 2011). In this regard, it has been observed that the minimal CA binding unit comprised of the CC-L2-SPRY domain of rhTRIM5α is sufficient to disrupt CA assemblies in vitro (Zhao et al., 2011). This suggests that the CA destabilizing activity is contained in this portion of the protein, even though no enzymatic activity has been ascribed to these domains of TRIM5α. The data here provide a biophysical explanation for this observation. If, for example, CC-L2-SPRY were to bind assembled CA in either of the more extended conformations we observe by smFRET, the transition to more compact conformations, driven by the formation of helices in the L2 region, may induce mechanical stress on the CA lattice that induces premature disassembly. In support of this model, we and others have identified mutations in rhTRIM5α which abrogate restriction without affecting CA binding (Sastri et al., 2014, 2010; Yang et al., 2014). Specifically, the RRV-275-277AAA mutant can bind CA but fails to restrict HIV-1. The reduced α-helical content associated with this mutant (Fig. 1, Sastri et al., 2014), taken together with the fact that it exclusively occupies the more extended FRET state (Fig. 4) is consistent with a model where the formation of L2 helix 3 may act as a spring which triggers the displacement of the neighboring SPRY domain. MD simulations of the rhTRIM5α SPRY domain binding to assembled CA suggest that the SPRY domain can intercalate deep into the threefold interhexameric cleft (Kovalskyy and Ivanov, 2014), potentially providing leverage that translates SPRY domain translocation to disruption of the three fold axis, as observed by Zhao et al. (2011). Although additional studies are required to demonstrate that the conformational changes in the CC-L2 dimer observed by smFRET might actually drive the CA disassembly observed by Zhou et al., the data provided here demonstrate that smFRET is a valuable technique with which to assess the conformational changes that might occur in the context of rhTRIM5 dimers bound to assembled CA.

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