Supporting Information

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SI Materials and Methods

Construction, Expression, and Purification of TRIM5-21R Proteins. Baculovirus expression vectors for One-STrEP-FLAG (OSF)-tagged TRIM5-21R proteins were based on the previously reported Gateway (Invitrogen) donor vector, pDONR221/OSFT-TRIM5-21R (WISP-08-177) (1). We first created a new vector that encoded full-length, wild-type TRIM5-21R (pDONR221/OSFP-TRIM5-21R, WISP-10-430), but had a PreScission protease site (LEVLFQGP) in place of the TEV protease site of WISP-08-177 and lacked an artifactual C-terminal Gly-Gly extension. Quikchange site-directed mutagenesis (Stratagene) was used to introduce the R121E mutation (rhesus TRIM5α numbering) into the B-box 2 domain (pDONR221/OSFP-TRIM5-$21R_{R121E}$, WISP-10-431) or to introduce a premature stop codon after residue 276 (pDONR221/OSFP-TRIM5-$21R_{1-276}$, WISP-10-432, encoding the ΔSPRY construct used for in vitro assembly). These constructs were incorporated into baculovirus expression vectors using the BaculoDirect system (Invitrogen) in SF21 cells. The recombinant baculovirus that encoded the ΔSPRY construct (OSFT-TRIM5-$21R_{1-232}$) used for the binding experiments illustrated in Fig. 3 was described previously (1).

Expression and purification methods for the new OSF-tagged PreScission-cleavable TRIM5-21R constructs were adapted from protocols described previously (1). SF21 cells (6 L at 2×10^6 cells/mL in SF-900 II serum-free media, Invitrogen) were infected with recombinant baculovirus at a multiplicity of infection of 4, and harvested by centrifugation 48 h later. All protein purification steps were performed at 4 °C. Cells were resuspended in lysis buffer [50 mM Tris, pH 8, 50 mM NaCl, 1 mM tris(2-carboxyethyl)phosphine (TCEP)] supplemented with 1.5% (w/v) Triton X-100 and 0.007 volume of protease inhibitor cocktail (Sigma), and lysed in a 100-mL Dounce homogenizer (15 strokes). The lysate was clarified by ultracentrifugation (184,000 × *g* for 50 min), filtered (0.45 μm), and loaded onto a 5-mL StrepTrap HP column (GE Lifesciences). The column was washed with 50 mL of lysis buffer and eluted in the same buffer supplemented with 2.5 mM D-desthiobiotin. The eluate was loaded onto two 5-mL HiTrap HP Q-Sepharose columns (GE Lifesciences) connected in series, and the protein was eluted with a 100-mL linear NaCl gradient (0.05–0.5 M). The TRIM5-21R monomers and dimers eluted as distinct peaks (1) that were collected separately and dialyzed into size-exclusion buffer (12 h, 20 mM Tris, pH 8, 25 mM NaCl, 1 mM TCEP). For in vitro assembly experiments (but not for CA binding assays), the affinity tag was removed during this dialysis step by the addition of 100 units of PreScission protease (GE Lifesciences) per mg of substrate. Proteins were then purified to homogeneity by size-exclusion chromatography in dialysis buffer (Superdex 200 16/60 column, GE Lifesciences), and concentrated using a Vivaspin 20 concentrator (30 kDa cutoff, Sartorius Stedim). At this stage, we noticed that there was an inverse relationship between protein solubility (assessed by the appearance of aggregates that induced light scattering at 340 nm) and assembly competence. For example, the assembly-competent wild-type TRIM5-21R dimer could be concentrated to only ∼1.5 mg/mL in size-exclusion buffer (pH 8), whereas the assembly-incompetent R121E mutant could be concentrated to ∼30 mg/mL in the same buffer. The TRIM5-$21R_{1-276}$ ΔSPRY construct and wild-type TRIM5-21R monomer had intermediate solubilities (∼2 mg/mL and ∼10 mg/mL, respectively).

The following modifications to the protocol were made for TRIM5-21R proteins used in the HIV-1 CA-NC cocrystallization experiments: (*i*) buffers contained 20 mM N-cyclohexyl-2-aminoethanesulfonic acid (CHES), pH 10 instead of Tris, (*ii*) NaCl was omitted, except during the gradient elution step of anion-exchange chromatography, and (*iii*) PreScission protease cleavage was performed in the anion-exchange eluate *prior* to dialysis into size-exclusion buffer. These modifications prevented premature protein assembly and also improved protein solubility slightly.

The masses of all purified TRIM5-21R proteins were within 2 Da of the expected values (assuming loss of N-terminal methionines and N-terminal acetylation), as determined by electrospray ionization mass spectrometry. Typical yields of TRIM5-21R dimers were ∼0.5 mg/L culture for the wild-type and TRIM5-$21R_{1-276}$ proteins, and ∼5 mg/L for TRIM5-$21R_{R121E}$. Yields of the monomeric proteins were typically ∼5-fold lower than the dimeric proteins.

Construction, Expression, and Purification of HIV-1 CA Proteins. The HIV-1 $CA_{A14C/E45C}$ protein used for binding experiments was expressed and purified as described previously (2, 3). The HIV-1 CA-$NC_{A14C/E45C/W184A}$ expression construct was created by Quikchange site-directed mutagenesis of WISP-96-18 (4), and the protein was expressed and purified as described (4), except that β-mercaptoethanol (βME) was added at a concentration of 100 mM to the lysis buffer, and maintained at 20 mM in all subsequent steps. The unusually high concentration of βME in the lysis buffer was empirically determined to improve yield, presumably by disrupting preassembled and/or aggregated protein (2, 3).

Binding Assays. Disulfide-stabilized HIV-1 CA tubes were assembled by stepwise dialysis of 1 mg/mL HIV-1 $CA_{A14C/E45C}$ as described previously (2). Purified OSF-tagged wild-type, R121E, and ΔSPRY TRIM5-21R proteins were incubated in the presence or absence of the disulfide-stabilized CA tubes for 1 h at 25 °C in binding buffer (20 mM Tris, pH 8, 25 mM NaCl, 1 mM TCEP), with CA subunits present in a 6-fold molar excess over TRIM5-21R subunits. Unbound TRIM and unassembled soluble CA proteins were separated from assembled and bound complexes by centrifugation through a 70% (w/v) sucrose cushion (in binding buffer lacking TCEP). At these low protein concentrations, unbound TRIM5-21R proteins did not assemble appreciably, and therefore remained in the supernatant fraction. Aliquots (200 μL) of the binding reactions were layered onto cushions (4 mL) and subjected to centrifugation at 108,000 × *g* for 30 min at 4 °C. Input and supernatant fractions were sampled directly. The protein pellet was dissolved in 200 μL of binding buffer, concentrated by precipitation with 5% (vol/vol) trichloroacetic acid, and resuspended in 10 μL of SDS-PAGE sample buffer supplemented with 2 μL of 2 M urea, 2 M Tris, pH 12.

Proteins were separated by SDS-PAGE, electrotransferred onto nitrocellulose membranes, and visualized by Western blotting with a rabbit anti-HIV-1 CA (made in-house, 1:5,000 dilution) or a mouse anti-FLAG antibody (Sigma,1:3,000 dilution) to detect OSF-tagged TRIM5-21R constructs. Secondary antibodies (1:20,000 Alexa 680-nm, Molecular Probes; or 1:10,000 IRDye 800-nm, Rockland) were detected using an Odyssey infrared imaging system (Li-Cor, Inc.).

1. Langelier CR, et al. (2008) Biochemical characterization of a recombinant TRIM5α protein that restricts human immunodeficiency virus type 1 replication. *J Virol* 82:11682–11694.
2. Pornillos O, et al. (2009) X-ray structures of the hexameric building block of the HIV capsid. *Cell* 137:1282–1292.
3. Pornillos O, Ganser-Pornillos BK, Banumathi S, Hua Y, Yeager M (2010) Disulfide bond stabilization of the hexameric capsomer of human immunodeficiency virus. *J Mol Biol* 481:985–995.
4. Ganser BK, Li S, Klishko VY, Finch JT, Sundquist WI (1999) Assembly and analysis of conical models for the HIV-1 core. *Science* 283:80–83.

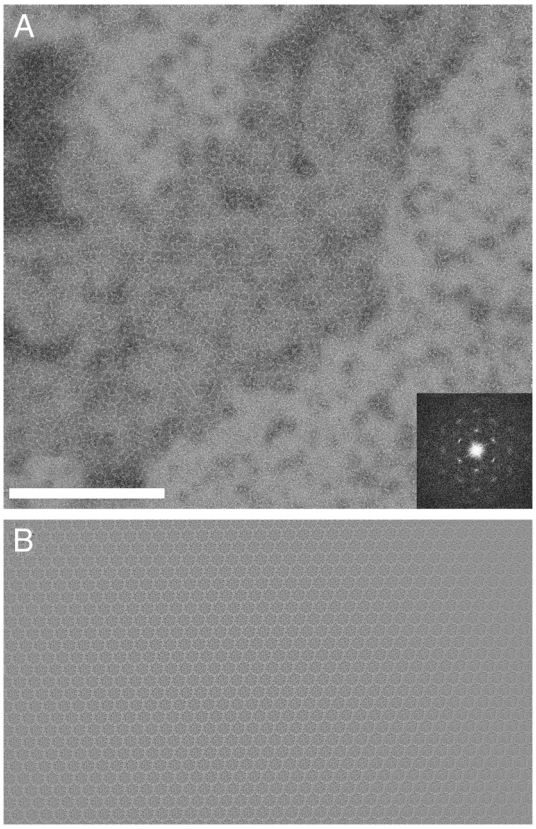

Fig. S1. Analysis of the hexagonal lattice formed by the truncated TRIM5-21R$_{1-276}$ (ΔSPRY) protein. (*A*) Image of the negatively-stained assemblies, with the computed Fourier transform of the central section (inset). (Scale bar,500 nm). (*B*) Fourier filtered image of the crystal shown in part (*A*).

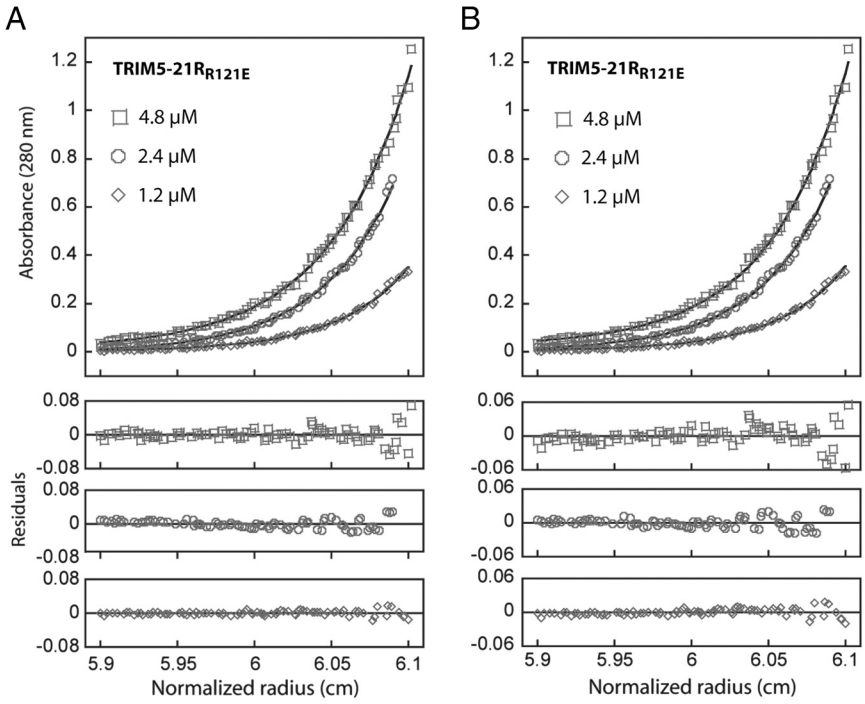

Fig. S2. Equilibrium sedimentation distributions of purified OSF-tagged TRIM5-21R$_{R121E}$. Centrifugation experiments were performed at 4 °C in 20 mM Tris, pH 8, 25 mM NaCl, 1 mM TCEP, using an Optima XL-I centrifuge and An-60 Ti rotor (Beckman). The data points (open symbols) and the best-fit curves (solid lines) are shown at the top, and residual differences between the data and two different single-species models are shown at the bottom. The data shown are for initial protein concentrations of 1.2, 2.4, and 4.8 μM at a rotor speed of 14,000 rpm. Data sets were also collected at 16,000 rpm, and all of the data were globally fitted to: (*A*) a model in which the molecular weight was not constrained during the refinement (MW_{calc} = 62,253 Da, MW_{obs} = 119,017 Da, MW_{obs}/MW_{calc} = 1.91) or (*B*) a model in which the molecular weight was fixed to that of a dimer (124,506 Da). Nonlinear least-squares data fitting was performed using the Heteroanalysis software (1). Solvent density and protein partial specific volume were calculated with the program SEDNTERP (2).

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2 Laue TM, Shah BD, Ridgeway TM, Pelletier SL (1992) Computer-aided interpretation of analytical sedimentation data for proteins. *Ultracentrifugation in Biochemistry and Polymer Science*, eds Harding SE, Rowe AJ, Horton JC (Royal Society of Chemistry, Cambridge, England), 90–125.