Supporting Information

Yang et al. 10.1073/pnas.1210903109

SI Materials and Methods

Protein Expression and Purification. The optimized truncation constructs of PRY/SPRY$_{rh}$ (residues 275–493) or PRY/SPRY$_{hu}$ (residues 273–489) was cloned into the expression vector pMAT9s (1) containing an N-terminal 6×His-tag followed by maltose-binding protein (MBP). Constructs were verified by DNA sequencing (The Keck DNA Sequencing Facility, Yale University, New Haven, CT). The expression plasmids were coexpressed with the chaperon expression plasmid pGro7 (Takara Mirus Bio) in BL21(DE3) *Escherichia coli* cells and induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 2.0 mg/mL L-arabinose at 18 °C overnight. The resulting MBP-fusion protein was purified by an MBPtrap HP affinity column (GE Healthcare), followed by a HitrapQ anion exchange column (GE Healthcare) in 50 mM Tris (pH 8.0) using a 10- to 150-mM NaCl gradient elution. MBP-PRY/SPRY was further purified using a HiLoad 16/60 Superdex 200 prep grade column (GE Healthcare) in 50 mM Tris (pH 8.0), 300 mM NaCl, and 0.1 mM Tris (2-carboxyethyl) phosphine (TCEP), and the protein purity was examined by SDS/PAGE. Protein was concentrated to 1.5 mg/mL for crystallization.

Crystallization and Data Collection. MBP-PRY/SPRY$_{rh}$ crystals were grown at 16 °C using the microbatch-under-oil and the hanging-drop vapor diffusion methods by mixing 0.4–1.8 μL protein containing 6% (wt/vol) glucose and 6% (wt/vol) trehalose with 1 μL of crystallization buffer containing 100 mM MES (pH 6.2) and 25% (wt/vol) PEG 3350. The best crystals were grown with protein concentration at 1.5 mg/mL. Crystals were cryo-protected by the crystallization buffer with 30% (vol/vol) glycerol and frozen in liquid nitrogen. Diffraction data were collected at the Advanced Photon Source beamline 24-ID and the National Synchrotron Light Source beamline X29A to the resolution of 3.3 Å. Data were processed using HKL2000 (2). The data statistics are summarized in Table S1.

Structure Determination and Refinement. There are two MBP-PRY/SPRY$_{rh}$ molecules in the asymmetric unit of the crystal. The structure was solved by molecular replacement using PHASER (3) with a ligand-binding MBP structure [Protein Data Bank (PDB) ID 1ANF] as the first search model. Phases calculated from the initial solution with MBP were improved only by noncrystallographic symmetry (NCS) averaging using RESOLVE (4) and DM (5). Clear electron density of PRY/SPRY$_{rh}$ was evident in the NCS-averaged map (Fig. S1). The solution for the PRY/SPRY domains was found by a real-space search in MOLREP (6) using the murine TRIM21 PRY/SPRY structure (PDB ID 2VOL) as the search model. The NCS-averaged electron density map was used for model building in the program Coot (7). Refinement was carried out with iterative rounds of Translation Libration Screw-motion (TLS) and restrained refinement using Refmac5 (8), followed by rebuilding the model to the 2Fo-Fc and the Fo-Fc maps using Coot. The final model has an R_{work}/R_{free} of 21.0%/24.8%. The refinement statistics are summarized in Table S1.

Structural Analysis and Illustrations. Pairwise superposition of a series of PRY/SPRY structures was performed using the program SHP (9). A full matrix of evolutionary distances was calculated, and the tree representation was generated using the program PHYLIP (10).

Binding Assays with Capsid Hexamers. Capsid (CA) protein (A14C/E45C/W184A/M185A) was expressed in *E. coli* and purified as described (11). For in vitro assembly, 1–2 mL of protein was dialyzed into 1 L of buffer (50 mM Tris·HCl, 1 M NaCl, 200 mM β-mercaptoethanol, pH 8 at 4 °C), followed by dialysis into 2 L of the same buffer lacking β-mercaptoethanol to permit formation of disulfide crosslinking within the hexameric rings. To recover hexamers, tubular assemblies were dissociated by dialysis into 50 mM Tris·HCl (pH 8). The product was >95% intact hexamer as determined by nonreducing SDS/PAGE and Commassie staining. Individual CA hexamers (CA concentration of 400 μM), PRY/SPRY$_{rh}$ (200 μM), and their mixture of the same protein concentrations were loaded onto a Superdex 200 HR 10/30 column (GE Healthcare) separately. The elution profiles were compared to monitor whether the complex was formed.

Binding Assays with CA Tubes. CA tubes were assembled from 80 μM (2 mg/mL) CA in 1 M NaCl and 50 mM Tris·HCl (pH 8.0) at 37 °C for 1 h. The binding buffer, 50 mM Tris·HCl (pH 8.0), 300 mM NaCl, and 0.1 mM TCEP, is also the stock buffer for the TRIM5α PRY/SPRY proteins. Briefly, binding buffer containing different concentrations of human or rhesus TRIM5α PRY/SPRY was added to 20 μL of preassembled CA tubes. The reaction mixture was incubated on a rocking platform at room temperature for 1 h with gentle mixing at 10-min intervals. At the end of incubation, 5-μL samples were withdrawn from the reaction mixtures and immediately used for cryo-electron microscopy (cryo-EM) analysis, and 0.5-μL samples from the same reaction mixtures were mixed with 4× lithium dodecyl sulfate (LDS) sample buffer (Invitrogen) supplemented with 10 mM DTT for SDS/PAGE analysis. The remaining sample was pelleted at 20,000 × *g* with an Eppendorf centrifuge 5417R for 30 min at 4 °C. Supernatants of 0.5 μL and 1.5-μL pellet samples [resuspended with a prespin volume of buffer containing 1 M NaCl, 50 mM Tris·HCl (pH 8.0)] were mixed with 4× LDS sample buffer for gel analysis. Total (t), supernatant (s), and pellet (p) samples, without boiling, were loaded on 10% SDS/PAGE and stained with InstantBlue (Expedeon). Each experiment was carried out at least three times.

Cryo-EM Analysis. Aliquots from the binding assays (above) were subjected to cryo-EM analysis. A total of 2.5 μL was applied to the carbon side of glow-discharged perforated Quantifoil grids (Quantifoil Micro Tools), and 3 μL of binding buffer was added to the backside of the grids. Grids were blotted and plunge-frozen into liquid ethane using a manual gravity plunger. Low dose (10 ∼15e^-/Å^2) projection images were collected with an FEI Tecnai Polara electron microscope at a nominal magnification of 4,700× and 59,000×, with underfocus values ranging from 1.0 to 2.5 μm, using a Gatan ultrascan 4K×4K CCD camera (Gatan Inc.).

1. Yang H, et al. (2010) Structural insight into the mechanisms of enveloped virus tethering by tetherin. *Proc Natl Acad Sci USA* 107(43):18428–18432.
2. Otwinowski Z, Minor W (1997) Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* 276:307–326.
3. McCoy AJ, et al. (2007) Phaser crystallographic software. *J Appl Cryst* 40(Pt 4):658–674.
4. Terwilliger TC (2000) Maximum-likelihood density modification. *Acta Crystallogr D Biol Crystallogr* 56(Pt 8):965–972.
5. Cowtan K (1994) 'dm': An automated procedure for phase improvement by density modification. *Joint CCP4 and ESF-EACBM Newsletter on Protein Crystallography* 31: 34–38.

6. Vagin A, Teplyakov A (2010) Molecular replacement with MOLREP. *Acta Crystallogr D Biol Crystallogr* 66(Pt 1):22–25.
7. Emsley P, Cowtan K (2004) Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* 60(Pt 12 Pt 1):2126–2132.
8. Vagin AA, et al. (2004) REFMAC5 dictionary: Organization of prior chemical knowledge and guidelines for its use. *Acta Crystallogr D Biol Crystallogr* 60(Pt 12 Pt 1):2184–2195.
9. Stuart DI, Levine M, Muirhead H, Stammers DK (1979) Crystal structure of cat muscle pyruvate kinase at a resolution of 2.6 A. *J Mol Biol* 134(1):109–142.
10. Felsenstein J (1997) An alternating least squares approach to inferring phylogenies from pairwise distances. *Systematic biology* 46(1):101–111.
11. Pornillos O, et al. (2009) X-ray structures of the hexameric building block of the HIV capsid. *Cell* 137(7):1282–1292.
12. Yap MW, Nisole S, Stoye JP (2005) A single amino acid change in the SPRY domain of human Trim5alpha leads to HIV-1 restriction. *Curr Biol* 15(1):73–78.
13. Li Y, Li X, Stremlau M, Lee M, Sodroski J (2006) Removal of arginine 332 allows human TRIM5alpha to bind human immunodeficiency virus capsids and to restrict infection. *J Virol* 80(14):6738–6744.
14. Pham QT, Bouchard A, Grütter MG, Berthoux L (2010) Generation of human TRIM5alpha mutants with high HIV-1 restriction activity. *Gene Ther* 17(7):859–871.
15. Maillard PV, Reynard S, Serhan F, Turelli P, Trono D (2007) Interfering residues narrow the spectrum of MLV restriction by human TRIM5alpha. *PLoS Pathog* 3(12): e200.
16. Sawyer SL, Wu LI, Emerman M, Malik HS (2005) Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. *Proc Natl Acad Sci USA* 102(8):2832–2837.
17. Sebastian S, et al. (2009) An invariant surface patch on the TRIM5alpha PRYSPRY domain is required for retroviral restriction but dispensable for capsid binding. *J Virol* 83(7):3365–3373.
18. Perron MJ, Stremlau M, Sodroski J (2006) Two surface-exposed elements of the B30.2/SPRY domain as potency determinants of N-tropic murine leukemia virus restriction by human TRIM5alpha. *J Virol* 80(11):5631–5636.
19. Arriagada G, Muntean LN, Goff SP (2011) SUMO-interacting motifs of human TRIM5α are important for antiviral activity. *PLoS Pathog* 7(4):e1002019.
20. Stremlau M, Perron M, Welikala S, Sodroski J (2005) Species-specific variation in the B30.2 (SPRY) domain of TRIM5alpha determines the potency of human immunodeficiency virus restriction. *J Virol* 79(5):3139–3145.
21. Byeon IJ, et al. (2009) Structural convergence between Cryo-EM and NMR reveals intersubunit interactions critical for HIV-1 capsid function. *Cell* 139(4):780–790.

A

B

C

MBP

PRY/SPRY$_{rh}$

Thr452

Phe455

Glu292

Arg 297

Fig. S1. (*A*) The overall structure of the MBP (gray)-PRY/SPRY$_{rh}$ (yellow). (*B*) Unbiased electron density of PRY/SPRY$_{rh}$ before its inclusion in the model. The electron density is from the twofold NCS-averaged 2fo-fc map (1σ), calculated using the molecular replacement solution with the MBP model only. (*C*) Details of the unbiased electron density.

Fig. S2. Superposition of the crystal structures of PRY/SPRY$_{rh}$ (yellow) onto human TRIM21 (green), GUSTAVUS (cyan), and human Ash2L (magenta), respectively. The statistics of the superposition (number of equivalences, distances, and rmsd values) are indicated below the images. The disordered region in rhesus TRIM5α (rhTRIM5α) is shown in black.

Fig. S3. Structural mapping of positive selection and mutagenesis hotspots (side chains in stick representation) in PRY/SPRY$_{rh}$ (backbone in ribbon representation). The missing loop (V1) is modeled and shown in black. The hotspot residues are grouped into five classes. Class 1 (green) includes human TRIM5α mutants that gain the ability to inhibit HIV-1, SIV, or B/NB-MLV (12–15). Class 2 (cyan) are hot spots under strongest selection during evolution (16). Class 3 (orange) is composed of residues important for N-tropic murine leukemia viruses (N-MLV) restriction, but not necessary for capsid binding (17). Class 4 (red) includes amino acids also involved in binding to N-MLV CA (18). Class 5 (magenta) includes SUMO-interacting motifs 1 and 2, which are important for N-MLV restriction activity of different TRIM5α orthologs (19). Mutations of the residues underlined (18, 19), in addition to the V1 loop (black) (16, 20), are implicated in loss of function of rhesus TRIM5α.

Fig. S4. (*A*) Superposition of the CA hexamers of HIV and MLV. (*B*) The surface regions implicated in the interaction with the Trim5α PRY/SPRY domains occupy the same locations in the HIV and MLV hexamer, although the HIV L4/5 loop is longer than its counterpart in MLV.

Fig. S5. Schematic depiction of the intrahexameric (*A*) and the interhexameric (*B*) CA binding of PRY/SPRY$_{rh}$ (red and yellow surface) along the different directions in HIV CA tubes (tan: N-terminal domain; light blue: C-terminal domain). The interfaces corresponding to the a-c, b-c, and a-b directions observed in the HIV CA helical tube (21) (Fig. 4*D*) are shown in the top three panels. The CA hexamer interface models are created by docking the crystal structure of the HIV CA hexamer to the EM map of the CA helical tube (Electron Microscopy Data Bank accession code: EMD-5136). Note that the angles/distances between dimeric PRY/SPRY$_{rh}$ changes in *A* and that the flexible V1 loop adopts different conformations in *B* to fit the varying curvatures. The maximum separation of CA hexamers at the narrow tip of the HIV capsid cone (~45° between hexamer axes) is shown at the bottom, where V1 reaches the maximum extension for binding in *B*.

Table S1. Data collection and refinement statistics

	$MBP\text{-}PRY/SPRY_{rh275}$
Data Collection	
X-ray source	X29 BNL
Wavelength (Å)	1.075
Space group	*C*2
Unit cell a,b,c (Å)	170.8, 98.7, 110.3
Unit cell α,β,γ (°)	90.0, 123.0, 90.0
Resolution range (Å)	50.0–3.30 (3.42–3.30)*
Unique reflections	22,400 (2,238)
Completeness (%)	96.5 (97.1)
Redundancy	3.0 (3.0)
$I/\sigma I$	6.69 (1.39)
R_{merge} †	0.166 (0.889)
Refinement	
Resolution range (Å)	49.4–3.30
No. of reflections (working/test)	21,248/1,135
R_{work}/R_{free}	0.210/0.248
Number of atoms	
Protein	8,746
Ligand	70
Water	44
B-factors	
Protein	86‡
Ligand	75‡
Water	49
r.m.s.d	
Bond lengths (Å)	0.010
Bond angles (°)	1.4

*Numbers in parentheses are for the highest resolution shell.

†$R_{merge} = \langle S_{hkl}\, S_j | I_j(hkl) - \langle I(hkl) \rangle | \rangle / \langle I(hkl) \rangle$, merging statistics for all symmetry mates.

‡TLS contributions added to the B-factors.