INTRODUCTION
Myxovirus resistance (Mx) proteins are important restriction factors in the interferon response against viruses (1). There are two isoforms in mammals, grouped into Mx1 (MxA)—like and Mx2 (MxB)—like proteins, based on evolutionary analysis (2). Although human MxA has been well documented to restrict a broad range of viruses, including influenza, vesicular stomatitis, and Thogoto (3), the antiviral activity of human MxB has only been recently discovered, decades after its initial identification (4) and characterization (5). Studies have shown strong inhibition of HIV-1 replication by ectopic (6–8) or endogenous expression (8) of MxB. MxB primarily targets the viral core after cell entry and after reverse transcription (9). In certain cell types, this interaction is dependent on the HIV-1 capsid protein (CA) host factor cyclophilin A (8, 10). MxB also blocks nuclear import of preintegration complexes and proviral integration (6, 11). Several primate MxB proteins have since been reported to show species-dependent variation in inhibiting the infection of lentiviruses (12). Naturally occurring HIV-1 CA mutations (13) and transmitted/founder virus strains (10) have also been found to escape inhibition by MxB, suggesting an active selective pressure on HIV-1 evolution.

Mx proteins belong to the dynamin superfamily of large guanosine triphosphatases (GTPases) (fig. S1), which share a core structure comprising a GTPase, a bundle signaling element (BSE), and a stalk domain (1). The structural homology among these proteins is exemplified in the crystal structures of human MxA (14) and human MxB lacking its N-terminal region (NTR) (Δ1–83) (15), where individual domains are practically superimposable (root mean square deviation of 0.8 to 1.1 Å) (15). Dynamin family proteins share common properties of self-assembly into ordered helical arrays and exhibiting guanosine triphosphate (GTP)-dependent assembly or disassembly (16). Although these properties, along with the L4 loop that confers antiviral specificity (17), were found to be important for MxA function, the L4 loop (11, 18) and GTPase activity (6, 7, 11) appear to be dispensable for MxB anti-HIV-1 activity. Instead, the antiviral activity of MxB requires its capsid binding NTR (6) and the ability to oligomerize (18, 19). However, no structural information is available for either the protein oligomers or the NTR, owing to difficulties with protein preparation of wild-type MxB. Current knowledge of protein oligomerization and assembly of the dynamin family is largely based on the cryo-electron microscopy (cryoEM) structure of dynamin-ΔPRD (deleted proline-rich domain) tubes at 12 Å resolution (20). Therefore, we sought to obtain essential structural information of MxB oligomers and to dissect the specific interfaces responsible for the protein’s anti-HIV-1 activity.

RESULTS AND DISCUSSION
Purification of full-length wild-type MxB
To obtain full-length wild-type MxB, we expressed and purified an N-terminal maltose binding protein (MBP) fusion protein from mammalian cells. MBP-MxB is purified as oligomers when eluted from a Sephacryl S-500 HR gel filtration column following amylose affinity chromatography (Fig. 1A) such that a gradient of different oligomeric species was observed (Fig. 1, B to E). Single-particle analysis and twodimensional (2D) classification of the negatively stained EM images from the main peak fraction (Fig. 1C) revealed that these MxB oligomers have various extents of packing (Fig. 1F), indicating that the sample was too heterogeneous for further structural analysis. Like other dynamin family members (16), MxB spontaneously assembled into highly ordered long helical tubes at 150 mM NaCl (Fig. 2A), even at low protein concentrations (0.05 mg/ml; 0.4 μM). Immunogold labeling localized the MBP fusion tag to the outer surface of the tube (Fig. 2B), suggesting that the MxB NTR is oriented toward its outer circumference. The helical assembly was not induced by the MBP tag because removal of the tag did not affect tube formation and instead induced tube bundling/aggregation (fig. S2).

CryoEM structure of MxB reveals a novel oligomerization interface critical for HIV restriction
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Human dynamin-like, interferon-induced myxovirus resistance 2 (Mx2 or MxB) is a potent HIV-1 inhibitor. Antiviral activity requires both the amino-terminal region of MxB and protein oligomerization, each of which has eluded structural determination due to difficulties in protein preparation. We report that maltose binding protein–fused, full-length wild-type MxB purifies as oligomers and further self-assembles into helical arrays in physiological salt. Guanosine triphosphate (GTP), but not guanosine diphosphate, binding results in array disassembly, whereas subsequent GTP hydrolysis allows its reformation. Using cryo-electron microscopy (cryoEM), we determined the MxB assembly structure at 4.6 Å resolution, representing the first near-atomic resolution structure in the mammalian dynamin superfamily. The structure revealed previously described and novel MxB assembly interfaces. Mutational analyses demonstrated a critical role for one of the novel interfaces in HIV-1 restriction.

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Effect of GTP binding on MxB helical assembly

MxB was previously shown to have GTPase activity in immunoprecipitates (5). We determined that purified MxB hydrolyzes GTP with the GTPase activity (k_{obs}) comparable to the basal GTPase hydrolysis rates of other dynamin-like proteins (fig. S3) (16). The addition of GTP (Fig. 2G and fig. S4B) or nonhydrolyzable GTP analogs, such as guanosine 5′-O-(3′-thiotriphosphate) (GTP-γS) or guanosine-5′-[(β,γ)-methylene] triphosphate (GMP-PCP) (Fig. 2, E and F), to the MxB tubes completely disrupted them, whereas the addition of guanosine diphosphate (GDP) (Fig. 2, C and D) or GTP without MgCl2 (fig. S4A) had no effect. Overnight treatment with GTP (Fig. 2H), but not with GMP-PCP or GTP-γS (Fig. 2F), resulted in MxB tube reassembly. These results suggest that GTP binding, but not hydrolysis, is sufficient to exert conformational changes that disrupt the MxB helical assembly, wherein upon hydrolysis, MxB reverts back to the assembly-competent conformation. It should be noted that although GTP-γ-S or GMP-PCP binding disassembles the MxB tubes or depolymerizes the MxA rings (21), binding of these GTP analogs, on the contrary, promotes helical assembly of other dynamin family members such as human dynamin 1 (22), yeast Dnm1 (23), and human Drp1 (24).

CryoEM structure of the MxB assembly

The assembled MxB tubes were highly ordered with an inner and outer diameter of 55 and 275 Å, respectively (Fig. 3A and fig. S5). There was an undifferentiated density out to 360 Å, as shown in one of the 2D classes (Fig. 3A, between white and blue dashed lines), presumably corresponding to the MxB NTR and MBP tag. The Fourier transforms of the MxB tubes indicated that they belong to a one-start helical family of (~6, 1) (fig. S5), a right-handed helix (fig. S6) with a rise of 8.237 Å and a twist of 58.4°. Using cryoEM and real-space helical reconstruction (25), we determined the 3D density map of the MxB helical assembly at 4.6 Å resolution (Fig. 3B and fig. S7). The local resolution of the density map varies (Fig. 3B); α-helical turns (fig. S8, D to G) and some bulky side-chain densities (fig. S8, D and G) are resolved at the inner core (stalk and BSE), whereas the GTPase domain appears to be more flexible. The NTR, together with the MBP tag, is not resolved, probably because of their flexibility. Initial rigid-body docking of individual domains from the crystal structure of the NTR-truncated MxB dimer [Protein Data Bank (PDB) ID: 4WHJ] (15) resulted in a reasonable overall fit, although it revealed substantial deviations, particularly at the first stalk helix S1 and unaccounted extra helical density (fig. S8A, arrows). This helical region is part of the L4 loop, which conveys antiviral specificity for MxA-like proteins (17). It was previously thought to be completely unstructured and was not observed in either the MxA (26) or MxB (15) crystal structure.

We modeled the L4 helix de novo using Rosetta into the MxB dimer structure and carried out molecular dynamics flexible fitting (MDFF), followed by real-space refinement to obtain an all-atom cryoEM structure model of the MxB assembly (Fig. 3, D and F, fig. S8, and movie S1). The resulting model displays a good match to the experimental density (Fig. 3D and fig. S8, C to G). In the cryoEM structure, the entire MxB assembly is made up of MxB dimer units. Six dimers go hand in hand, interlocking with each other through the stalk and BSE domains to form one rung, where the sixth dimer comes around to interact with the first dimer, forming the one-start right-handed helix (Fig. 3, F and G, and movie S2). The tube surface displays a shallow groove, where the GTPase domains cluster, and a deep groove, where the NTRs are presumably located (Fig. 3, F and G). The MxB dimer in the assembly is substantially different from the crystal dimer (PDB ID: 4WHJ), displaying (i) a highly kinked and extended stalk S1 helix C terminus (Ss1c),...
which effectively displaces the base of S\(_{1c}\) by 27°, (ii) a shift in the domain orientations about hinge 1 between the BSE and the stalk by 22°, and (iii) a well-ordered L4 loop helix (Fig. 3E). As a result, these new features led to the formation of completely new assembly interfaces, which are distinct from those previously inferred from crystal contacts (table S1) (discussed in detail below). There is no swapped dimer present, as previously thought for the dynamin tube (20).

**Novel MxB assembly interfaces**

The cryoEM structure revealed three levels of assembly interfaces that are likely shared by members of the dynamin superfamily of GTPases: dimer interface [interface 2; following the MxA convention (26)], oligomer interfaces (interfaces 1 and 3), and helical (higher-order) assembly interface (interface 4) (Figs. 3G and 4, A and B). The dimer interface (interface 2) is essentially the same, as seen in the MxB crystal structure (fig. S9A). However, the other three interfaces are novel in the cryoEM structure, as described in detail below.

Interface 1 is formed by the symmetric interaction of the tip of the stalk domain (S\(_{1n}\)) of one dimer and the BSE domain (B\(_{1c}\)) from another, mediated by a salt bridge (D417–K693) and hydrophobic contacts (F420, M419, and I696) (Fig. 4C and fig. S9B). This interface is markedly different from the putative interface 1 (fig. S10A and table S1) gleaned from the crystallographic symmetries of MxA and MxB structures, which shows stacking of the stalk domains (S\(_{1n}\) and S\(_{1c}\)) of the dimers to form a linear array (14, 15).

Interface 3 is completely novel and composed of the new extended base of kinked S\(_{1c}\) and its connecting loops at the either end of S\(_{1c}\), L1 and L2 (Fig. 4D and fig. S9C). Conserved hydrophobic L2 residues (F495 and V496) from adjacent dimers form a symmetric interaction (Fig. 4D, blue and orange, and fig. S9C). Additional salt bridges, R449–F495 and V496 from adjacent dimers form a symmetric interaction L1 and L2 (Fig. 4D and fig. S9C). Conserved hydrophobic L2 residues of the dimers to form a linear array (14, 15).

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**Functional importance of MxB interface 3 in assembly and antiviral activity**

With detailed knowledge of all interfaces responsible for assembly, we tested which of these interfaces is relevant to the MxB function. Because the dimer interface was previously well characterized for its important role in MxB antiviral function (15, 18, 19), we focused...
on the three new assembly interfaces: interfaces 1, 3, and 4. Representative residues at these interfaces were substituted to evaluate their effects on the ability of MxB to form oligomers or assemble into tubes and to restrict HIV-1 in cultured cells. Mutations across these interfaces—F420D in interface 1, F495D and R449D in interface 3, and E285K in interface 4—diminished MxB tube formation (Fig. 4F). Although interface 1 and 4 mutants retained the ability to form oligomers greater than a dimer, only interface 3 mutants (F495D and R449D) failed to oligomerize (Fig. 4F). Coincidentally, only interface 3 mutations (F495D, R449D, and E484K) appreciably affected the anti–HIV-1 activity of MxB (Fig. 4G). Substitution of R455, which forms a salt bridge with E484 in our structure (Fig. 4D and fig. S9C), was previously found to disrupt HIV-1 restriction by MxB (table S1) (11, 18). Correlating the biochemical results with infection data strongly suggests that nonhelical MxB oligomers greater than a dimer are the active anti–HIV-1 species and that the new interface 3 is critical for MxB oligomerization and antiviral function. Given the prevailing GTP concentrations in the cell (~0.47 mM) (30), MxB is likely GTP-bound and not helically assembled. Although the structure of the MxB NTR remains elusive, our near-atomic resolution cryoEM structure of assembled, wild-type MxB allows for hypothetical models of possible MxB-capsid interactions, wherein an MxB tetramer could potentially recognize the mature capsid lattice, thus precluding the need for GTPase activity and interface 4 for MxB anti–HIV-1 activity (31, 32). Further, the high-resolution structure of a member of dynamin superfamily and the detailed molecular interactions responsible for GTP-dependent assembly or disassembly provide a structural framework for the function of other dynamin family GTPases. Dynamins generally catalyze GTP hydrolysis through the dimerization of GTPase domains (16), and in the context of helical assembly, this dimerization interface (GG interface) is thought to form between rungs (33). The GG interface is completely absent in the MxB helical assembly, and the conformation of the BSE domains of the helical MxB relative to the GTPase domain may represent the “closed” conformation, similar to that observed in the GDP-bound or apo crystal structure. Dynamins generally catalyze GTP hydrolysis through the dimerization of GTPase domains (16), and in the context of helical assembly, this dimerization interface (GG interface) is thought to form between rungs (33). The GG interface is completely absent in the MxB helical assembly, and the conformation of the BSE domains of the helical MxB relative to the GTPase domain may represent the “closed” conformation, similar to that observed in the GDP-bound or apo crystal structure. 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Fig. 3. CryoEM structure of the MxB helical assembly. (A) 2D class average of MBP-MxB helical segments from cryoEM images. Dashed white and blue lines indicate the outer surfaces of the MxB density and MBP density, respectively. Scale bar, 10 nm. (B) CryoEM reconstruction of an MxB tube shown in surface rendering, contoured at 4.5σ, and viewed parallel (left) and perpendicular (right) to the tube axis. Color indicates the local resolution of the density map ranging from 4.25 Å (blue) to 5.25 Å (red). The locations of the domains are indicated. (C) Domain structure of MxB. (D) Density map (contoured at 3σ) of an MxB dimeric assembly unit overlaid with a real-space refinement model of the MxB dimer. Domains are color-coded as in (C). Arrows point to the new L4 helix (purple). (E) Superposition of the cryoEM model and the crystal structure of MxB monomer (PDB ID: 4WHJ) (yellow). Arrows indicate the differences between the two structures: hinge 1, kinked extended stalk helix Sα1, and the new helix in L4. Inset, enlarged view of the boxed region. Dashed lines and red arrows show the change in helix orientation. (F) Atomic model of the MxB assembly in one rung, comprising six MxB dimers (1 to 6), each uniquely colored. Surface grooves are marked. (G) Schematic of the MxB helical assembly, hierarchically through three levels: a dimer, a rung, and a tube.


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We speculate that the trans-dimerization of the GTPase domains upon GTP binding of MxB is sensed by the BSE domains, causing them to transition to the open conformation, which effectively disrupts the helical assemblies.

**MATERIALS AND METHODS**

**Plasmid construction**

Cloning vectors containing the gene for the full-length wild-type human MxB (UniProt ID: P20592-1) and the MBP tag were gifts from J. Ahn (Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine). The expression vector pcDNA3.1(+) was obtained from Life Technologies (Invitrogen). The MxB gene and the MBP tag were amplified by polymerase chain reaction (PCR) and then subcloned, using the NEBuilder HiFi Assembly kit (New England Biolabs Inc.), into pcDNA3.1(+) that had been linearized by the restriction enzymes Eco RV and Xba I. The resultant insert, designated as MBP-MxB-H6, has a leading Kozak sequence, an N-terminal MBP tag, followed by a human rhinovirus 3C protease cut site, the full-length wild-type MxB, and a C-terminal hexahistidine tag (H6). The plasmid used for the infectivity assay was generated by transferring the PCR construct for MBP-MxB-H6 or MxB-hemagglutinin from pcDNA3.1(+) to pIRES2–enhanced green fluorescent protein (eGFP) (34). The sequences of the inserts were confirmed by DNA sequencing (Genewiz Inc.).

**Sequence alignment**

Amino acid sequences of Mx and dynamin proteins were obtained from UniProt using the indicated IDs and were then aligned using MUSCLE (35). The alignment was visualized using ESPript3 (36).
Expression and purification of MxB
Recombinant MxB was transiently expressed in mammalian cells using the Expi293 Expression kit from Life Technologies (Invitrogen). Suspension-adapted Expi293 cells were grown in Expi293 Expression Medium to a density of 3.5 × 10^6 cells/ml. Cells were then incubated at 37°C and 150 mM NaCl, 10 mM MgCl2, 2 mM dithiothreitol (DTT), 4 mM phenol, and 1 mM GTP. MxB was added just before measurement. The decrease in NADH absorbance at 340 nm was monitored in a 96-well plate using a Waters Q-ToF Premier, and peptides were identified using ProteinLynx Global Server.

Electron microscopy
Sample preparation
To prepare samples for initial screening by negative stain, the elution from the amylose resin was immediately filtered through a HiPrep Sephacryl S-500 HR (GE Healthcare) in buffer A with an additional 2 mM DTT. To induce the formation of the long tubes, the elution from the amylose resin was diluted in assembly buffer [20 mM Heps-KOH (pH 7), 150 mM NaCl, 1 mM MgCl2, 2 mM EGTA, and 2 mM DTT] at 0.5 to 1 mg/ml, and then incubated for the indicated period of time.

Negative stain EM
Aliquots (3 µl) from the gel filtration samples or the helical assembly were adsorbed to a glow-discharged, 400-mesh, carbon-coated copper grid and stained with fresh uranyl formate (2%). Images were recorded on a TF20 electron microscope (FEI) equipped with a field-emission gun at the indicated magnification on a 4k × 4k Gatan UltraScan charge-coupled device camera (Gatan).

Immunogold labeling
Immunogold labeling, modified from the study by Mears et al. (38), was performed to determine the orientation of the NTR of MxB in its helical form. Samples containing the MxB tubes were prepared and applied to a grid, as described above. The grid was successively floated on the following solutions: (i) twice with blocking buffer [bovine serum albumin (BSA;10 mg/ml) in oligomerization buffer] for 5 min, (ii) with blocking buffer containing primary antibody against MBP tag (Abcam) (1:250 dilution) for 1 hour, (iii) twice with blocking buffer for 5 min, and (iv) with blocking buffer containing a 5-mm gold-labeled secondary antibody (Ted Pella) (1:250 dilution) for 1 hour. All incubations were carried out at 4°C, and the grid was washed once with blocking buffer and twice with oligomerization buffer before staining with uranyl formate.

CryoEM
Three microliters of the MxB tubes (0.5 mg/ml) was applied on the carbon side of glow-discharged holey R2/1 Quantifoil grids (Quantifoil Micro Tools GmbH), manually blotted from the backside, and then plunge-frozen in liquid ethane using a homemade manual plunger. Images were collected under low-dose conditions (~40 e⁻/Å² total) using a Polara 300-kV microscope with a field-emission gun and an FEI Falcon II detector. Movies (~1000, each with seven frames) were manually collected using SerialEM (39) at a nominal magnification of ×98,000 (1.147 Å/pixel), with under-focus values ranging from 1.5 to 3.5 µm.

Image processing and helical reconstruction
Movie frames were aligned using UCSF (University of California, San Francisco) MotionCorr v2.1 (40), and the resulting motion-corrected sums were used for contrast transfer function estimation using Gctf v0.50 (41). Micrographs were then sorted on the basis of image quality, and ~630 micrographs were used for subsequent helical reconstruction using RELION 2.0 beta (25), which is a software package in development that integrates a helical processing workflow. Helical segments were boxed using EMAN 2.0 helixboxer.py (42), and diffraction patterns from individual tubes or 2D class averages generated using Spring (43) were used to estimate the helical parameters. A small data set with 3486 segments (500-pixel box size) was used in the helical processing workflow in RELION 2.0 beta to refine the helical parameters, which corrected mass spectra were acquired in MS² (all-ion) mode using a Waters Q-ToF Premier, and peptides were identified using ProteinLynx Global Server.
converged to a rotation angle of 58.4° and a rise of 8.25 Å. Using the previously generated 2D classes as templates, helical filaments were automatically picked from the full data set, resulting in 51,535 segments (450-pixel box size, 90.41% overlap between neighboring boxes, and interbox distance six times the helical rise). The segments were then analyzed by 2D classification, and 64 of 100 classes showing clear structural details were then selected for further processing (44,955 segments correspond to ~270,000 subunits). The first round of refinement, using a featureless cylinder (236 pixels in diameter and low-pass–filtered to 30 Å), resulted in a map with a resolution of 6.9 Å. For the next round of refinement, the previous reconstruction was used as an initial reference and converged to a map with 6.6 Å overall resolution, which was improved to 5.0 Å after postprocessing (sharpening and application of soft-edged mask). A final round of refinement was performed using polished particles, which gave a map with 5.3 Å resolution. Postprocessing by soft-edge masking and B-factor sharpening resulted in a map with 4.6 Å resolution. The final map was then low-pass–filtered, according to the local resolution estimated using RELION 2.0. Further 3D classification did not reveal distinguishable reconstructions, suggesting that there was no mixing of helical symmetry in the data set. Attempts to resolve the outer portion of the tube by 3D classification using cylindrical masks (150 to 360 Å in diameter) did not show any improvement to the features of the outer portion of the map.

Infectivity assay

Human embryonic kidney (HEK) 293T cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, penicillin (100 IU/ml), and streptomycin (0.1 mg/ml). For virus production, cells were plated 1 day before transfection. Single-round HIV-1 harboring the gene for firefly luciferase (HIV-Luc) was generated by cotransfecting HEK293T with pNLX.Luc.(R–)ΔAvrII and pCG-VSV-G (44). The virus yield was assessed by HIV-1 CA p24 enzyme-linked immunosorbent assay (ABL Inc.).

Before infection, HEK293T cells were transiently transfected with parental pIRES2-eGFP vector or derivatives expressing MxB. At 24 hours after transfection, GFP-positive cells selected by fluorescence-activated cell sorting were infected in duplicate with HIV-Luc (0.1 pg of p24 per cell) in the presence of polybrene (4 μg/ml). Forty-eight hours after infection, cells were lysed, and luciferase activity was determined as described (34, 44). Luciferase values were normalized to the level of total proteins in cell lysates, as determined using a bicinchoninic acid (BCA) assay (Pierce).

Western blotting

Cells were lysed in 50 mM tris-HCl (pH 8), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS, and total protein concentration was determined using the BCA assay. Samples (10 μg of total protein) were separated by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and reacted with goat polyclonal antibody to MxB (N-17), followed by HRP-conjugated secondary bovine anti-goat antibody (Santa Cruz Biotechnology). As an internal control, HRP-conjugated antibody to β-actin (Sigma-Aldrich) was used. Membranes were developed using the ECL Prime reagent (Amersham Biosciences) and imaged with a ChemiDoc MP imager (Bio-Rad).

De novo structure modeling of the L4 helix (residues 579 to 598)

Structure modeling of the L4 helix, which was apparent in the cryoEM density map but was missing in the crystal structure (PDB ID: 4WHJ), was modeled using Rosetta (45). Fragments containing sequence 3-mers and 9-mers were generated using the Rosetta server, using the MxB wild-type sequence (46). One thousand candidate folds were generated using the TopologyBroker protocol in RosettaScripts (47). The structural variability of the predicted folds was modest because all the predicted structures corresponded to an extended α-helix. Using the Talaris2014 potential (48), the lowest energy fold was selected for further refinement, as explained in the following sections.

Molecular dynamics flexible fitting

The x-ray–derived structure of the MxB dimer (PDB ID: 4WHJ) was used as the initial model. Missing loops were modeled using Modeller v9.17 (49). In addition, residues 487 to 490, which contain four alanine mutations in the structure (PDB: 4WHJ), were reverted back to the wild-type sequence. For the initial modeling of full-length MxB, the L4 helix was omitted, and residues 577 and 626 were treated as C-terminal and N-terminal, respectively, to cap the ends. Secondary structural assignments used as structural restraints (ss-restraints) during the MDFF protocol (50) were derived using a secondary structure prediction program, DSSP (51). To preserve the cis/trans conformations present in the initial model (PDB ID: 4WHJ), cis-peptide restraints were also used. The model was then subjected to MDFF, with the backbone coupled to the experimental density, with a coupling constant ramping from 0.05 to 0.15 over 5 ns, resulting in a cross-correlation between the map and the structure of 0.85. Because of the ss-restraints, the fitting of the stalk helix So1c into the experimental density was poor; therefore, the ss-restraints for residues 472 to 474 were manually removed. Subsequently, the model was subjected to MDFF, with a coupling constant ramping from 0.05 to 0.15 over 5 ns, resulting in a cross-correlation of 0.86. All molecular dynamics simulations were performed using NAMD 2.10 (52), with an integration time step of 2 fs, bonded interactions computed every time step, and electrostatics updated every 4 fs. Particle mesh Ewald was used for long-range electrostatics with a grid size of 1 Å. The CHARMM36 force field (53) and the TIP3P water model (54) were used in all simulations.

Iterative refinement of the MxB dimer by Rosetta and MDFF

The MDFF-derived MxB dimer model was further refined using Rosetta by following a similar procedure as the one developed by Lindert and McCammon (55). In particular, the MxB model was refined using the CartesianSampler available in RosettaScripts. For this purpose, the de novo model of L4 helix was docked into the experimental density using UCSF Chimera (56). First, a hybrid model of dimeric MxB containing the docked L4 helix and the MDFF-derived MxB dimer was obtained using Modeller (49). The hybrid model was then subjected to further refinement using the CartesianSampler in RosettaScripts (47). The model, which consists of the MDFF-derived MxB dimer and the Rosetta-derived model for residues 579 to 598, was further refined using MDFF, with a density coupling ramping from 0.05 to 0.15 over 5 ns.

Modeling of the MxB helical assembly

Using UCSF Chimera, starting from the Rosetta-MDFF MxB dimer, an entire MxB helical assembly was constructed by using a helical symmetry with a rise of 8.327 Å and an angle of 58.4°. The helical model was refined using MDFF with symmetry restraints (57). In addition, fitting of L1 and L2 into the density was further improved by using the interactive MDFF protocol with a coupling between the backbone atoms and a density of 0.1 (50). The interactive MDFF protocol manually guides the backbone of the loops into the experimental density.
The final cross-correlation between the helical model and the density map was 0.92.

**Molecular dynamics simulations and analysis**

An equilibrium molecular dynamics simulation of the entire MxB tube was performed starting from the refined helical MDFF-Rosetta model. The molecular dynamics simulations were performed using NAMD 2.10 (52), with an integration time step of 2 fs, bonded interactions computed every time step, and electrostatics updated every 4 fs. Particle mesh Ewald was used for long-range electrostatics with a grid size of 1 Å. The CHARMM36 force field (53) and the TIP3P (54) water model were used in all simulations. Analysis of salt bridges, hydrogen bonds, and hydrophobic contacts was performed in VMD (visual molecular dynamics) (58) and averaged over all interfaces of the tube. A probability score was assigned to each contact, based on the occupancy of the contact in the assembled structure during the molecular dynamics simulation.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/9/e1701264/DC1

table S1. Effects of MxB mutations at intermolecular interfaces 1, 3, and 4.

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fig. S9. Enlarged views of the intermolecular interfaces in the MBxl assembly.

fig. S10. Comparison of interfaces between cryoEM and crystal structures.

fig. S11. Comparison of L1 and L2 contacts of MBxl and dynamin 3.

fig. S12. Interface 4 mutations relative to GTP-binding site.

movie S1. Molecular dynamics flexible fitting of MxB into the cryoEM map.

movie S2. MxB assemblies into a helical array.

movie S3. Structured helix kinks from linear to tubular array.

movie S4. Formation of Interface 3 during helical assembly.

**REFERENCES AND NOTES**


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Supplementary Materials for

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The PDF file includes:

- table S1. Effects of MxB mutations at intermolecular interfaces 1, 3, and 4.
- fig. S1. Sequence alignment of Mx and dynamin proteins.
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- fig. S13. Comparison of MxB and MxA GTPase-BSE domains.

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/3/9/e1701264/DC1)

- movie S1 (.mp4 format). Molecular dynamics flexible fitting of MxB into the cryoEM map.
- movie S2 (.mp4 format). MxB assembles into a helical array.
- movie S3 (.mp4 format). Sα1c helix kinks from linear to tubular array.
- movie S4 (.mp4 format). Formation of Interface 3 during helical assembly.
Table S1. Effects of MxB mutations at intermolecular interfaces 1, 3, and 4.

<table>
<thead>
<tr>
<th>Interface</th>
<th>Mutation</th>
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<td>&gt;250kDa&lt;sup&gt;st&lt;/sup&gt;</td>
<td>(18)</td>
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</table>

*Fold defect in HIV-1 restriction compared to the wild-type. 2x effect means 50% of wild-type antiviral activity. #Measurement of oligomerization by cross-linking (xl) or gel filtration (gf) or electron microscopy (em). †Mutations were thought to be at the hypothetical “Interface 3” (18) (fig. S8B). Mutations in bold are from this study and those in red have significantly reduced effect on anti-HIV-1 activity of MxB (cut off at < 3 fold effective compared to the wild-type). ND, not determined.
**fig. S1. Sequence alignment of Mx and dynamin proteins.** Amino acid sequences of Mx and dynamin proteins were aligned using MUSCLE and the alignment visualized using ESPript3. The sequences include: MX2_HUMAN (Uniprot ID P20592), MX1_HUMAN (P20591), MX1_MOUSE (P09922), MX2_MOUSE (Q9WVP9), MX2_PIG (A7VK00), MX_CHICKEN (Q90597), MXA_ZEBRAFISH (Q8JH68), DYN1_HUMAN (Q05193), DYN2_HUMAN (P50570), DYN3-3_HUMAN (Q9UQ16), DNM1L_HUMAN (O00429) and DNM1_YEAST (P54861). Residues with a conservation of greater than 70% are color-coded (D, E in red; R, K, H in blue; N, Q, S, T in grey; L, I, V, F, Y, W, M, C in green; P, G in brown). Alpha-helices are shown as cylinders, while beta-sheets as arrows, with colors as in Figure 3C. The N-terminal region is represented as red dashed line and the unresolved regions in black dashed line.

Residues involved in the interfaces (1, 3 and 4) are indicated on top of the alignment in bold (black in this study, red for MxB crystal contacts, 4WHJ) or at the bottom underlined (for dynamin 3 tetramer, 5A3F) and italicized (for MxA crystal contacts, 3SZR).
**fig. S2.** MxB assembles into helical tubes with and without the MBP tag. (A) Coomassie-stained SDS-PAGE gel and corresponding western blots, with the indicated primary antibody or probe. MBP-MxB-H6 was treated as indicated with HRV-3C protease at 150 mM NaCl. Proteins are indicated by arrows on the right. Dashed arrow points to a minor species of MxB with both MBP and the linker between MBP and MxB removed. (B&C) Negative stain EM images of samples in (A) without the protease (B) and with the protease (C). Removal of the MBP-tag resulted in ordered, but largely aggregated, MxB tubes. Scale bar, 50 nm.
fig. S3. GTPase activity of MBP-MxB at different protein and salt concentrations. GTPase activities on the left were measured at a constant MxB concentration of 1.4 µM, and on the right were measured at a constant NaCl concentration of 150 mM. GTPase activities of wt MxB were assayed using a continuous NADH-coupled assay at 1 mM GTP and 37°C. Each gray bar represents the mean of three independent measurements while the error bars represent the 95% confidence interval. MxB T151A at 1.3 µM concentration and 150 mM NaCl is included as negative control.
fig. S4. MBP-MxB tubes in the presence of GTP without or with MgCl₂. Negative-stain projection image of MBP-MxB helical assembly after incubating with assembly buffer for 2 hours in the presence of GTP without (A) and with MgCl₂ (B). Scale bar, 100 nm.
**fig. S5. CryoEM of the MBP-MxB assembly.** (A) A low-dose projection image of MBP-MxB tubular assembly. Scale bar, 50 nm. (B) A 2D class average of MxB tubes. (C) Fourier transform of the class average in B. The tube belongs to a 1-start helical family of (-6, 1). Helical indices are shown. Blue arrows point to the two vectors (1,0; -6) and (0,1;1) in helical indexing. Black arrow points to the layer-line at 16 Å resolution. (D) A Fourier transform of a raw image of MBP-MxB tube.
**fig. S6. Handedness of the MxB helical assembly map.** (A) Docking of the crystal structure of dimer MxB (PDB:4WHJ, yellow) into the right-handed MBP-MxB helical assembly map. (B) Docking of the crystal structure of dimer MxB into the left-handed map.
fig. S7. Gold-standard Fourier shell correlation curve of the MBP-MxB density map. The overall resolution is 4.6 Å using an FSC cut-off of 0.143.
fig. S8. MDFF and real-space refinement of the MxB helical assembly model. (A) Rigid body docking of individual domains of the MxB crystal structure (4WHJ). Arrows point to densities that do not fit. (B) Time-evolution of the Cα-RMSD between the MDFF simulation and the initial MxB dimer model; the initial model was built based on the MxB crystal-structure. Inset: time-evolution of the Cα-RMSD for the refinement of the MDFF-derived tubular model; the MDFF model is refined by applying a series of gaussian-smoothed electron-densities with sigma decreasing from 5 to 0 Å in 1 Å steps. Note the difference in the scale. (C) FSC curves between the cryo-EM density map and the molecular models of MxB GTPase-BSE domain (red), Stalk domain (blue), dimer (black), tube (purple), and rigid body docking of individual domains of the crystal structure (dashed black). (D to F) Representative views of the fitting of the refined model into the density map: stalk helices (Sα1, Sα2 and Sα3, along with bulky amino acids Y508 and F572) (D), stalk helices (Sα3 and Sα4) and loops (L3, L4 helix) (E), and clear helical density in L4 (F). The extra unfitted density in (E) is from the other monomer.
(G) A clipped view shows densities of bulky amino acid side chains (F539, F429, F531, F458, F461 and F572).
fig. S9. Enlarged views of the intermolecular interfaces in the MxB assembly. (A) Overlay of the MxB crystal dimer (grey) and the cryoEM dimer (orange) at the dimer interface, Interface 2. (B to D) Detailed view of the Interface 1 (B), Interface 3 (C) and Interface 4 (D). Specific residues at the interfaces are labeled.
**fig. S10. Comparison of interfaces between cryoEM and crystal structures.** (A) Putative interface 1 at crystal contacts (4WHJ) (left), Interface 1 in the cryoEM assembly (middle) and overlay of the two interfaces (cryoEM gold/green, 4WHJ, light blue). The intermolecular interactions are entirely different. (B) Hypothetical Interface 3 (26) (no interaction) in the crystal structure (4WHJ) (left), extensive interactions at Interface 3 in the cryoEM assembly (middle), and overlay of the interfaces (cryoEM gold/green, 4WHJ, light blue). Amino acids involved in the interfaces are shown. Alpha helices and loops are labeled. (C) Connection of two adjacent MxB dimers in linear crystal arrays (left), cryoEM curved assembly (middle) with each monomer colored individually, excluding G domains for clarity, and overlay of crystal (light blue) and cryoEM (gold/green) (right), showing completely different dimer/dimer organization.
fig. S11. Comparison of L1 and L2 contacts of MxB and dynamin 3. (A to B) Tetramers of MxB (A, orange and blue) and dynamin 3 (5A3F, B, cyan and yellow) show different degrees of curvature (black dashed line). Only the stalk domains are shown for clarity. The dimers are individually colored. (C) The stalk tetramers were superposed using the blue (MxB) and yellow (dynamin 3) dimer pairs, which aligned with RMSD of 1.146 Å. The orange and cyan dimers clearly do not overlay well. (D) Superposition of MxB and dynamin 3 at Interface 3 at L1. Residues from MxB are shown. Differences between MxB and dynamin 3 are observed. (E) Superposition of MxB and dynamin 3 at Interface 3 residues in L2, wherein a similar interface is seen between MxB and dynamin 3. Residues from both MxB and dynamin 3 are shown.
fig. S12. Interface 4 mutations relative to GTP-binding site. (A to D) MBP-MxB-T151A has been shown to bind GTPγS (28) but is unable to hydrolyze GTP. MBP-MxB-T151A self-assembled into ordered tubular structures at 150 mM NaCl (A) or in the presence of GDP (D), loosened tubes in the presence of GTP (B) or GTPγS. Scale bar, 50 nm. (E) GTPase domain of MxB is shown, with residues at Interface 4 (P284, E285 and K250) located at the opposite side of GTP binding site (T151) labeled.
fig. S13. Comparison of MxB and MxA GTPase-BSE domains. MxB GTPase-BSE domains (orange) are overlaid with the corresponding domains in MxA (cyan) in GMP-PCP bound “open” state (4P4S, left) and MxA in GDP-bound “closed” state (4P4T, right).