Structural and functional studies on the extracellular domain of BST2/tetherin in reduced and oxidized conformations

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HIV-1 and other enveloped viruses can be restricted by a host cellular protein called BST2/tetherin that prevents release of budded viruses from the cell surface. Mature BST2 contains a small cytosolic region, a predicted transmembrane helix, and an extracellular domain with a C-terminal GPI anchor. To advance understanding of BST2 function, we have determined a 2.6 Å crystal structure of the extracellular domain of the bacterially expressed recombinant human protein, residues 47–152, under reducing conditions. The structure forms a single long helix that associates as a parallel dimeric coiled coil over its C-terminal two-thirds, while the N-terminal third forms an antiparallel four-helix bundle with another dimer, creating a global tetramer. We also report the 3.45 Å resolution structure of BST2(51-151) prepared by expression as a secreted protein in HEK293T cells. This oxidized construct forms a dimer in the crystal that is superimposable with the reduced protein over the C-terminal two-thirds of the molecule, and its N terminus suggests pronounced flexibility. Hydrodynamic data demonstrated that BST2 formed a stable tetramer under reducing conditions and a dimer when oxidized to form disulfide bonds. A mutation that selectively disrupted the tetramer (L70D) increased protein expression modestly but only reduced antiviral activity by approximately threefold. Our data raise the possibility that BST2 may function as a tetramer at some stage, such as during trafficking, and strongly support a model in which the primary functional state of BST2 is a parallel disulfide-bound coiled coil that displays flexibility toward its N terminus.

Viral infection can induce a type I interferon response, which in turn stimulates the expression of many genes that encode innate immunity factors (1). One of the upregulated proteins, bone marrow stromal cell antigen 2 (BST2/tetherin/CD317/HM1.24), inhibits the release of HIV-1 and other enveloped viruses from the surface of cells in which it is expressed (2–10). HIV-1 virions retained at the cell surface in the presence of BST2 are fully formed and mature but remain attached to the cell surface by a bridge in which the extracellular domain of BST2 functions, with the leading model being that virions are held at the cell surface by a bridge in which the extracellular domain of one or, more likely, multiple molecules of BST2 spans the gap between plasma membrane and the membrane of the otherwise detached viral particle (13, 14, 34). It has been argued that the GPI modification targets BST2 to cholesterol-rich regions of the plasma membrane (33), which may concentrate the protein at sites of viral budding. However, removal of the GPI anchor reportedly does not release virions from their tethered state (14). Finally, functions for BST2, independent of viral restriction, have also been proposed in trafficking and signaling (33), and in organization of the subapical actin cytoskeleton (35).

It has been proposed that BST2 forms a parallel dimeric coiled coil that is stabilized by CS3–CS5, C63–C63, and C91–C91 disulfide bonds. This model is supported by nonreducing SDS/PAGE analyses of mutant proteins isolated from the surface of HEK293T cells (32, 34) and by a recent crystal structure of residues 89–147 of the BST2 extracellular domain (36). Both the N-terminal TM helix and the C-terminal GPI can be incorporated into HIV-1 virions, and it has therefore been suggested that BST2 either forms an antiparallel dimer (14) or that it forms a parallel dimer whose tethering orientation is not critical for viral restriction (34). We also note that the importance of BST2 dimerization has been disputed (37). Remarkably, HIV-1 restriction is preserved (at approximately 10% levels) in cells that express an engineered protein that was designed to maintain the overall BST2 architecture but does not conserve the specific amino acid residue identities (34). Finally, the efficiency of BST2-mediated HIV-1 inhibition appears to be concentration-dependent, because virions that contain BST2 can be released under...
conditions of low BST2 expression (34). These considerations emphasize the importance of determining the oligomeric state(s) of BST2. In an effort to understand the mechanistic basis for BST2 activity better, we have determined crystal structures and solution oligomerization states of oxidized and reduced BST2 proteins and tested the restriction activity of a structure-based mutant that cannot switch oligomerization states when reduced.

Results

Reduced BST2 Crystal Structure. We determined a structure of the human BST2 extracellular domain (residues 47-152) produced in Escherichia coli and crystallized in the presence of reductant to 2.6 Å resolution and R/Resolution values of 26.0%/27.8% (Fig. 1 and Table S1). All but a few of the BST2 residues at the termini of the four molecules in the asymmetric unit were visible, although the central regions (residues 92–117) have relatively weak density and high B values.

Subunits are arranged as parallel dimers that splay apart slightly to form an antiparallel 4-helix bundle over the N-terminal 40 residues and generate a 245 Å-long tetramer in the asymmetric unit (Fig. 1A). The remaining two-thirds forms a dimeric coiled coil, with residues 118–150 displaying the standard “knobs-into-holes” packing of a and d heptad repeat residues seen in classical coiled coils (38–40), and residues 92–117 packing more loosely to display some but not all of the a and d residue knobs-into-holes interactions (Fig. S1). Equivalent packing for the C-terminal two-thirds was reported by Hinz et al. (36).

Although each of the subunits forms one continuous helix that is devoid of sharp turns, the helices do not all superpose closely over their entire length. The two parallel dimers in the asymmetric unit are very similar to each other and superpose with a root mean square deviation of 0.79 Å over all Cα atoms (Fig. 1B), but the two subunits within one parallel dimer show an approximately 30° deflection in the vicinity of residue 90, near the tetramer-dimer transition (Fig. 1C). This asymmetry results from small differences spread over several residues and may indicate that the BST2 sequence encodes an asymmetric dimeric structure, although we prefer the explanation that the molecule has inherent flexibility.

CS3 and CS6 are contained within the N-terminal four-helix bundle while C91 is immediately C-terminal to the bundle. All three cysteines are reduced in this structure, whereas biochemical data indicate that BST2 forms disulfide bonds in the oxidizing environment of the cell exterior (34). Residues CS3 and CS6 are too far from their partners to form disulfide bonds without disrupting packing of the four-helix bundle region. In contrast, neighboring C91 residues could form a disulfide bond without substantial movement of their main chains from the crystal structure. We have built C91 residues with their sulfur atoms separated by 4.7 Å in this structure because the density, which is relatively poor in this region, does not indicate formation of a disulfide.

Oligomeric States in Solution. Equilibrium analytical ultracentrifugation (AUC) was used to determine the association state of recombinant BST2 proteins in solution. This method provides shape-independent estimates of mass and is therefore ideally suited for studies of highly extended assemblies such as BST2. These studies employed slightly longer BST2(47–154) constructs in order to include two tyrosine residues and thereby increase optical absorbance. The AUC data showed that the recombinant protein is a tetramer in solution under the reducing conditions used in crystallization, over an initial concentration range of 0.5–2.0 mg/ml (41.7–166.7 μM). Equilibrium protein distributions could be globally fit to a single species tetramer model (Fig. S2) and also indicated a tetrameric association when the molecular weight was allowed to float (Fig. 2A).

To determine if oligomerization was altered by oxidation, purified reduced BST2 was dialyzed for one week against identical buffer that lacked reductant. Mass spectrometry showed that this protein was a fully disulfide-linked dimer (Fig. S3). The protein eluted from a sizing column primarily as a well-defined peak but also included a broad peak at shorter retention times that presumably corresponded to oxidized aggregates. AUC showed that protein from the main peak is dimeric over the concentration range tested (0.5–2.0 mg/ml), either by fitting to a dimer (Fig. S2) or by allowing the molecular weight to float (Fig. 2B). These data indicate that BST2 forms a stable tetramer under reducing conditions and converts into a stable dimer upon oxidation.

Oxidized Crystal Structure. To determine the BST2 extracellular domain structure under oxidizing conditions, we expressed secreted BST2(51–151) in HEK293T cells and completed purification and crystallization in the absence of reductant. Crystals of Endo-H treated BST2(51–151) diffracted anisotropically to a maximum resolution of 3.45 Å resolution. This structure, with one dimer in the asymmetric unit, was determined by molecular replacement using residues 89–151 of the reduced structure as a search model. The refined model (R/Rfree = 26.9%/29.8%) comprises residues 72–151 for one of the two molecules in the asymmetric unit and residues 77–151 for the other molecule. The oxidized and reduced structures overlap closely from residue 90 to the C terminus but diverge toward the N terminus where the reduced protein tetramerizes (Fig. 3). For example, the Cβ–Cβ distance of juxtaposed A88 residues, which occupy an a coiled coil position, is 4.2 Å in the oxidized structure and 7.4 Å in the reduced structure. Clear electron density, confirmed by kicked omit maps, is observed for a disulfide bridge linking C91 residues validating the oxidized state of the molecule (Fig. 3). Two sugar moieties extending from N92 and positioned perpendicular to the C91–C91 disulfide could be located in the electron density (Fig. S4). Finally, additional N-terminal residues could be modeled in a helical conformation (Fig. S5), although due to the modest resolution, gaps in the density, and appearance of disorder, we have not included those residues in the deposited model. Thus, the dimeric coiled coil extends into the region that is tetrameric in the reduced structure, and residual density suggests that the coiled coil continues most of the way to the N terminus of this construct, albeit with pronounced flexibility.

Fig. 1. Structure of reduced BST2(47–152). (A) The four molecules in the asymmetric unit. Yellow/magenta and blue/green subunits form parallel dimers. (B) Overlap of the two parallel dimers. (C) Overlap of the blue and green subunits on residues 90–152. (D) Orthogonal views showing the L70 side chain buried at the center of the tetramer.
The Role of the BST2 Tetramer in HIV-1 Restriction. Although the functional importance of many BST2 residues has been tested by mutagenesis (34), the tetramer has not been reported previously. To create a mutant protein that was defective in tetramerization, we mutated Leu70, which lies at the heart of the four-helix bundle (Fig. 1D). Mutation to Asp destabilized the tetramer, so that the BST2(147–154)L70D protein remained dimeric, even under reducing conditions (Fig. 2 and Fig. S6). Thus, this mutation allowed us to test the restriction activity of a protein that dimerized but did not detectably tetramerize in vitro.

To assay restriction, HEK293T cells (which do not express BST2) were cotransfected with expression constructs for wild-type or mutant BST2 and an HIV-1NL4-3 proviral expression construct that lacked Vpu (HIV-1ΔVpu). BST2(L70D) expression was modestly increased (∼1.5-fold) across a series of different concentrations of expression construct (Fig. 4, “Cell” bottom panel). Virus released from cells that expressed wild-type and mutant BST2 was analyzed by (i) Western blotting of virion-associated MA and CA proteins in the culture media (Fig. 4, “Virus”) and (ii) measuring the infectious titers of released virions (Fig. 4B). These data showed that both the wild-type and mutant BST2 proteins were effective restriction factors because they reduced the release of infectious viral particles more than 5,000-fold when expressed at high levels. However, the restriction potency of BST2(L70D) was consistently about half that of the wild-type protein, despite higher expression levels (Fig. 4). The strong but attenuated activity of BST2(L70D) indicates that BST2 tetramerization is not essential, although it remains possible that it may contribute to the potency of viral restriction.

Discussion

We have determined crystal structures of the extracellular domain of BST2 under reducing and oxidizing conditions. The C-terminal two-thirds of the structure forms a parallel dimeric coiled coil. The C-terminal third adopts a classical knobs-into-holes packing, while the central third is less well-defined. At the N-terminal third of the protein, the two parallel helices splay apart to form an antiparallel four-helix bundle in the reduced state, but when the protein is oxidized these helices continue, at least initially, as a dimeric coiled coil that is stabilized by a C91–C91 disulfide and probably also by C53–C53 and C63–C63 disulfides. AUC data indicated that the crystal structures recapitulate the predominant solution oligomerization states because the reduced protein forms a stable tetramer but becomes dimeric upon oxidation (Fig. 2). The latter observation is consistent with an earlier study, which concluded that BST2 forms a disulfide-crosslinked dimer on the cell surface (34). Our data therefore indicate that BST2 can tether budded virions to the plasma membrane as a disulfide-linked coiled coil dimer.
The model in which BST2 forms a coiled coil from residue 47 to 151 predicts an overall length of 140 Å. This compares with the finding from SAXS measurements that the BST2(47–159) construct has an overall length of 170 Å (36). This 30 Å length difference might be explained by extended conformation(s) for residues 153–159, which are not present in any of the crystal structures plus a few additional residues at the ends of the molecule, which tend to be poorly defined in electron density. Partial unwinding of the N-terminal disulfide-bound portion of the coiled coils is also a possibility. A number of observations indicate that the N-terminal region of the BST2 extracellular domain is functionally important, including the findings that activity is lost when all disulfide bonds are disrupted (34) or when seven residues within 62–73 are mutated (36). Although we find that the L70D protein retains activity, thereby indicating that formation of a stable reduced tetramer is not essential for viral restriction, the restriction activity is reduced by approximately twofold compared to wild-type, even though expression of the mutant increases slightly. This reduction in potency indicates that L70 contributes to restriction, likely by stabilizing a functional conformation. Indeed, the activity reduction in the L70D mutant approaches the 10-fold loss of activity seen in an “artificial” BST2 molecule that replaces essentially all of the amino acid residues (34). L70D may diminish restriction activity by impacting the conformation or stability of the parallel coiled coil conformation. Alternatively, L70D may diminish activity by inhibiting formation of the reduced BST2 tetramer that, though not absolutely essential for HIV-1 restriction, might contribute to the efficiency of restriction or to some other BST2 function. It is striking that BST2 forms a stable tetramer in solution, indicating that the four-helix bundle is an energetically stable conformation. It is striking that BST2 forms a stable tetramer in solution, indicating that the four-helix bundle is an energetically stable conformation. It is striking that BST2 forms a stable tetramer in solution, indicating that the four-helix bundle is an energetically stable conformation. It is striking that BST2 forms a stable tetramer in solution, indicating that the four-helix bundle is an energetically stable conformation. It is striking that BST2 forms a stable tetramer in solution, indicating that the four-helix bundle is an energetically stable conformation.
sufficient for activity. There are a number of possible explanations, which are not mutually exclusive. Consistent with a report that the GPI anchor is dispensable for restriction (14), one possibility is that virions can be tethered by the two TM helices of a dimer embedding separately in plasma and virion membranes. A second possibility is that dimerization provides a more structured extracellular domain that is better able to fold and resist proteolysis. Third, formation of an extended coiled coil, rather than a more flexible isolated single subunit, may optimally separate the TM helices and GPI anchors, thereby increasing the chance that virions will bud between them and become tethered to the cell. Finally, dimerization may strengthen the tether through avidity effects that result from having two attachments to each membrane. Avidity effects may also explain why higher concentrations of BST2 molecules produce more efficient virion retention (34) because higher order BST2 dimer–dimer interactions could be stabilized in the constrained environment of the cell surface. Although important questions remain regarding the structure of the tether that connects virus and cell, our data indicate that a BST2 parallel coiled coil is likely to be a fundamental component.

**Methods**

**Structure Determination of Reduced BST2.** Human BST2(47–152) was expressed in E. coli, purified under reducing conditions, and concentrated to 9–16 mg/ml for crystallization (see SI Text for details). Crystals grew in sitting drops comprising 2 μl of protein solution and 2 μl of reservoir solution (18% PEG-MME 2K, 0.1 M BisTris pH 7.2, 4 μM TCEP). Crystals were transferred to reservoir solution made up with 30% MPD and plunged into liquid nitrogen. Data were collected from a selenomethionine-substituted crystal at SSRL beamline 7.2 and processed with HKL2000 (45). Eight of the expected twelve Se sites were located using the auto solve option in Phenix (46) and phased to 3.0 Å resolution. The model was built using COOT (47) and refined to 2.6 Å with REFMACS (48, 49) using translation/library/screw (TLS) parameters (50) and programs in the CCP4 suite (51). The crystallized construct includes six additional residues (GIDPFT) at the N terminus that are not visible in electron density maps. The following residues lack defined density: molecule A (K47); B (K152); C (K47, K152); D (K47, A48, N49, K151, K152).

**Structure Determination of Oxidized BST2.** Human BST2(251–151) was expressed in HEK293T cells, purified, and concentrated to approximately 80 mg/ml (Bradford) for crystallization (see SI Text for details). MRC crystallization plate sitting drops were set up using a Mosquito crystallization robot by mixing 100 nl of protein solution with an equivalent amount of reservoir. Drops comprising 2 μl of reservoir solution made up with 30% MPD and plunged into liquid nitrogen. Data were collected from a selenomethionine-substituted crystal at SSRL beamline 7.2 and processed with HKL2000 (45). Eight of the expected twelve Se sites were located using the auto solve option in Phenix (46) and phased to 3.0 Å resolution. The model was built using COOT (47) and refined to 2.6 Å with REFMACS (48, 49) using translation/library/screw (TLS) parameters (50) and programs in the CCP4 suite (51). The crystallized construct includes six additional residues (GIDPFT) at the N terminus that are not visible in electron density maps. The following residues lack defined density: molecule A (K47); B (K152); C (K47, K152); D (K47, A48, N49, K151, K152).

**Analogue Equilibrum Ultracentrifugation.** Data were collected at 4 °C in a T-optical, A-refractometer (Kontron). Reduced BST2(47–154) samples were in a buffer containing 20 mM Tris pH 7.4, 300 mM NaCl, and 4 mM TCEP. Alternatively, BST2(47–154) was oxidized by extensive (1 week) dialysis against the same buffer lacking reductant. Formation of a disulfide-linked dimer upon oxidation was verified by mass spectrometry (Fig. S3). Oxidized and reduced protein samples were centrifuged at 12,000 rpm and 16,000 rpm with initial protein concentrations of 2.1, 1.06, and 0.53 mg/ml (166.7 μM, 83.3 μM, 41.7 μM, respectively). Data were globally fit to ideal single species models with fixed or floating molecular masses using the non-linear least squares algorithms in the HETEROANALYSIS software (57). Protein partial specific volumes and solvent densities were calculated with the program SEDNTERP (version 1.09) (58).

**BST2 Restriction of Virus Release and Infectivity.** 293T and HeLa-TZM reporter cells obtained from Drs. J. C. Kappes and X. Wu through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH were maintained using standard procedures. Wild-type and mutant BST2 proteins were expressed from a pCAG vector under the control of a constitutively active CMV promoter. The HIV-1ΔVpu version of the HIV-1 molecular clone NL-3 has been described previously (12). Virus was produced by calcium phosphate transfection of semiconfluent six-well plates of 293T cells with 1 μg viral DNA and with increasing quantities of vectors expressing wild-type (BST2) or BST2Δ1844 (G1844 A) or BST2(2,5)Δ1844 (G1844 A, 25, 50, 100, 200, and 500 ng) or an empty pCAG control vector.

For Western blotting experiments, virus-containing media was harvested 40 h posttransfection. Virions (1 ml) were pelleted through a 20% sucrose cushion at 15,000 × g, and the pellet was resuspended in 30 μl 1X SDS/PAGE loading buffer (12 μl loaded/lane). Cells from the same samples were harvested, pelleted, lysed in 60 μl SDS-loading buffer, and boiled for 10 min. Lysates were loaded/lane. Protein samples were centrifuged at 12,000 rpm and 16,000 rpm with initial protein concentrations of 2 μg/ml (Bradford) for crystallization (see SI Text for details). MRC crystallization plate sitting drops were set up using a Mosquito crystallization robot by mixing 100 nl of protein solution with an equivalent amount of reservoir. Stacks of plate-like crystals grew using a reservoir of 8% PEG8000, 100 mM Tris-HCl pH 8.5 at 18 °C. Crystals were vitrified in crystallization reservoir supplemented with 25% glycerol for data collection at the Diamond Light Source I04 beamline. Data were integrated and scaled using the programs MOSFLM (52) and SCALA (53) of the CCP4 suite. The structure of BST2(251–151) was determined by molecular replacement using PHASER (54) and residues 89–151 of reduced BST2(47–152) as the search model. 20 cycles of rigid-body refinement were followed by 10 cycles of positional refinement using REFMACS (48, 49) with the occupancy of C91 residues set to 0.01. 2mFo–DFc and mFo–DFc maps indicated the existence of the C91–C91 disulfide and helical density was visible N-terminal to the template. Model building used COOT (47). Final refinement cycles used secondary structure restraints between hydrogen bonded helical N-O atoms. Phenix.refine (55) was used to generate secondary structure restraints and to calculate maximum likelihood averaged kick out maps (56). The refined model lacks a substantial portion of the N terminus, which could not be reliably built, although residual density is present, particularly in proximity to neighboring molecules in the lattice (Fig. S5).

**Schubert et al.**


Expression and Purification of Reduced BST2. Human BST2(47–152) was cloned into pET151/D-TOPO (Invitrogen) and expressed in BL21(DE3)RIL cells using the autoinduction technique (1). Ni-NTA affinity chromatography, dialysis against 20 mM Tris, pH 8.0, 100 mM NaCl, 2 mM DTT, and cleavage of the His-tag with TEV protease overnight at room temperature, was followed by Q (Buffer A: 20 mM Tris 8.8, 10 mM NaCl, 1 mM DTT; Buffer B: 20 mM Tris 8.8, 1 M NaCl, 1 mM DTT) and size-exclusion chromatography in 20 mM HEPES pH 7.0, 100 mM NaCl, 2 mM DTT. Protein was concentrated to 9–16 mg/ml for crystallization. Identical procedures were used for BST2(47–154) and selenomethionine-substituted BST2(47–152).

Expression and Purification of Oxidized BST2. Human BST2(51–151) was cloned into a pHLsec vector (a kind gift of Dr. Aricescu, Oxford) between the AgeI and KpnI cloning sites with a C-terminal GTKH₆ tag. After secretion signal cleavage, an EGT tripeptide is left at the N terminus resulting in the final EGT-(BST2 E51–K151)-GTKH₆ protein product [BST2(51–151)]. Transient protein expression was performed in HEK293T cells essentially as described (2). To facilitate protein deglycosylation, the N-glycosylation inhibitor swainsonine was added at a final concentration of 20 μM during DNA-PEI complex formation (3). Four days after transfection, the supernatant was collected for protein purification under nonreducing conditions. Following overnight binding to a His-Trap column (GE Healthcare), BST2(51–151) was eluted in 50 mM phosphate buffer pH 7.5, 300 mM NaCl with a linear (10–300 mM) imidazole gradient. The protein was then dialyzed against 50 mM Tris-HCl buffer, 50 mM NaCl with pH adjusted to 5.5 with a 1 M sodium citrate solution prior to overnight deglycosylation at 37 °C with Endoglycosidase-H (New England Biosciences) according to the manufacturer’s instructions. Deglycosylated BST2(51–151) was further purified by size-exclusion chromatography on a S200 16/60 column (GE Healthcare) in 50 mM Tris-HCl pH 7.5, 100 mM NaCl buffer.


**Fig. S1.** Sequence alignment with knobs and holes analysis. An alignment of representative sequences with secondary structure observed in the reduced structure shown above. The mature human protein is truncated at Ser160, the site of GPI anchor attachment. The glycosylation sites, Asn65 and Asn92, are indicated with blue dots and are solvent exposed in the crystal structures. Residues that display α or δ knobs into holes packing, as defined by the program SOCKET (1), are indicated with orange dots. Residues that are invariant in this alignment are highlighted in red boxes, and residues that are highly conserved are shown in red.

Fig. 52. Analytical equilibrium ultracentrifugation analyses of BST2(47–154). Equilibrium distributions without (A) and with (B) reductant (4 mM TCEP). Residual differences between the data and the fits are shown below. Rotor speeds were 12,000 rpm in these experiments. Data sets were also collected at 16,000 rpm. Data were collected at three concentrations and were globally fit to single species models in which the molecular weights were fixed to either a tetramer (left panel; 51,100 Da) or dimer (right panel, 25,550 Da). Note that the reduced BST2 protein is well fit by the tetramer model and the oxidized protein is well fit by the dimer model.
Fig. S3. Mass spectrometry of reduced and oxidized BST2(47–154). (A) Reduced and (B) oxidized BST2(47–154) were desalted for electrospray ionization mass spectrometry using a C18 Ziptip (Millipore) and analyzed on a Quattro-II mass spectrometer (Micromass, Inc.). Data were acquired with a cone voltage of 50 eV, a spray voltage of 2.8 kV, and scanning from 800 to 1,400 m/z in 4 s. Spectra were combined, and the multiply charged molecular ions were deconvoluted into a molecular-mass spectrum by using MaxEnt software (Micromass, Inc.). The mass of the reduced species corresponded to a BST2(47–154) monomer (MW_{obs} = 12,774.6 g/mol, MW_{calc} = 12,775.4 g/mol), indicating no disulfide formation in the presence of reductant. In contrast, the majority of BST2(47–154) formed a disulfide crosslinked dimer in the absence of TCEP (MW_{obs} = 25,544.0 g/mol, MW_{calc} = 25544.8 g/mol, assuming formation of three disulfide bonds).
Fig. S4. Density showing glycosylation of N92 in BST2(51–151) expressed in HEK293T cells. $2mF_o - DF_c$ electron density ($1.0\sigma$, blue) indicates the position of discernible sugar moieties extending from N92 residues after Endo-H treatment. N-acetylglucosamine (NAG) residues are approximately perpendicular to the C91–C91 disulfide bond.

Fig. S5. Residual electron density at the N terminus of oxidized BST2(51–151). (A) Density in the $2mF_o - DF_c$ (blue, $1.0\sigma$) and $mF_o - DF_c$ (green, $3.0\sigma$) maps is visible at the N terminus of BST2(51–151) (orange). It is mostly evident in proximity to symmetry related molecules (gray). (B) Residual density suggests the possibility of alternative helical conformation for the N-terminus, which we have tentatively modeled in this figure. Flexibility at the N terminus could generate alternative coiled-coil dimers shown here in cyan and yellow, respectively.
Fig. S6. Analytical equilibrium ultracentrifugation analyses of wt BST2(47–154)L70D. Equilibrium sedimentation distributions of BST2(47–154)L70D with reductant (4 mM TCEP). Corresponding residual differences are shown below. Rotor speeds for the data shown here were 12,000 rpm. Data sets were also collected at 16,000 rpm. Data were collected at three concentrations, and all of the data were globally fit to single species models in which the molecular weight was allowed to float (left panel; MW_{obs} = 26,908 Da), fixed as a dimer (middle panel; 25,554 Da) and fixed as a tetramer (right panel; 51,108 Da). Note that the reduced BST2(47–154) L70D protein is well fit by the dimer model.
Table S1. Data collection and crystallographic refinement statistics

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<td>Favored (%)</td>
<td>98</td>
<td>98.04</td>
</tr>
<tr>
<td>Allowed (%)</td>
<td>2.0</td>
<td>1.96</td>
</tr>
<tr>
<td>Disallowed (%)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Values in parentheses are for the highest-resolution shell.

*R_{sym} = \sum|I-I|/\sum I, where \langle I\rangle is the average intensity from multiple observations of equivalent reflections.

*R_{factor} = 100 \times \sum |F_o-F_c|/\sum |F_o|$. R_{free} is the R_{factor} computed from the 7.6% of reflections in the case of 3nwh and 4.5% in the case of 2xg7 that were chosen randomly and excluded from the refinement calculations.