Table S1. Coordination geometry of the metal-binding site

Ligands	Bond length (Å)				
	ssA2	**ssA2-D1596A**	**ssA2-N1602A**	**ssA2-D1596A/N1602A**	**wtA2**
A1600 O	2.3	2.9	2.8	3.0	2.7
R1597 O	2.3	2.5	3.1	3.3	2.8
D1498 O_δ	2.3	2.7	3.0	3.0	3.3
D1596 O_δ	2.4	-	2.5	-	-
N1602 O_δ	2.3	2.5	-	-	2.9
Water 1	2.4	2.7	3.4	2.4	3.0
Water 2	-	-	-	-	3.1

Figure S1. Close-up views of the metal binding site. (A) Superposed ssA2 and wtA2 structures. The structure of ssA2 is colored in cyan, and that of wtA2 (PDB code 3GXB) in pink. The metal ion in the ssA2 structure is shown as a golden sphere. The new disulfide bond of ssA2 and the vicinal disulfide bond of wtA2 are shown with ball-and-stick models. **(B)** Superposed composite omit map and *Fo-Fc* simulated annealing omit map of the bound metal ion and the surrounding residues. The composite omit map contoured at 1.0 σ and the *Fo-Fc* simulated annealing omit map contoured at 2.0 σ are colored in gray and green, respectively. The residues surrounding the bound metal ion are shown with ball-and-stick models. The metal ion and a nearby water molecule are shown in golden and red spheres, respectively.

Figure S2. The anomalous dispersion analyses. (A) Ca^{2+} binding in the ssA2 structure. The anomalous difference Fourier map (3.0 σ contour level) of the Ca^{2+} ion in the ssA2 structure in complex with Ca^{2+} is shown in yellow. **(B)** Superposition of the structures of ssA2 with or without treatment of EDTA. The structure of the EDTA-treated ssA2 (green) is very similar to that of the untreated ssA2 (cyan) except that no calcium signal could be detected in the anomalous dispersion difference analysis. A water molecule which coordinates the Ca^{2+} ion in the ssA2-Ca^{2+} structure (cyan) and the equivalent one in the uncomplexed ssA2 (green) are colored accordingly.

Figure S3. Relative positions of the glycosylation sites and the metal binding site. The ssA2 (cyan) and wtA2 (pink) structures are superposed. The glycosylation sites and the Ca^{2+}-binding site are located on opposite sides of the A2 domain with a distance of about 30 Å in between. The glycosylation appears to have no effect on the conformations of the structural elements at or around the glycosylation sites, nor the Ca^{2+}-binding site. In the ssA2 structure, the Ca^{2+} ion is

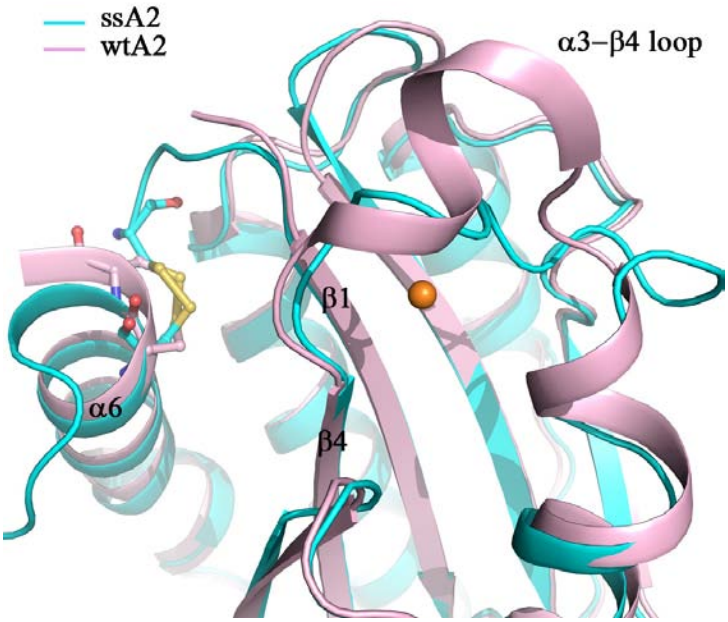
shown in golden sphere, and the coordinating residues are shown with ball-and-stick models. In the wtA2 structure, glycosylated Gln1515 and Gln1574 are shown with ball-and-stick models and colored in magenta.

Figure S4. Crystal packing analyses. (A) Crystal packing analysis of wtA2 (PDB code 3GXB). The α3-β4 region (circled) of a wtA2 molecule (pink) is involved in crystal packing with two symmetry-related wtA2 molecules (wheat). **(B)** Superposition of the ssA2 structure with the wtA2 structure. The superimposed ssA2 structure is colored in cyan. In the crystal packing of wtA2, the α3-β4 region of ssA2 would clash with the symmetry-related molecule, which is denoted with a circle.

Figure S5. A close-up view of the α3-β4 loop and the β4 strand of ssA2. The α3-β4 loop, the β4 strand, and the other structure elements are colored in yellow, green, and cyan, respectively. The residues on the α3-β4 loop which are involved in Ca^{2+} binding are shown with ball-and-stick models.

Figure S1

A


ssA2
wtA2
α3−β4 loop
β1
α6
β4

B

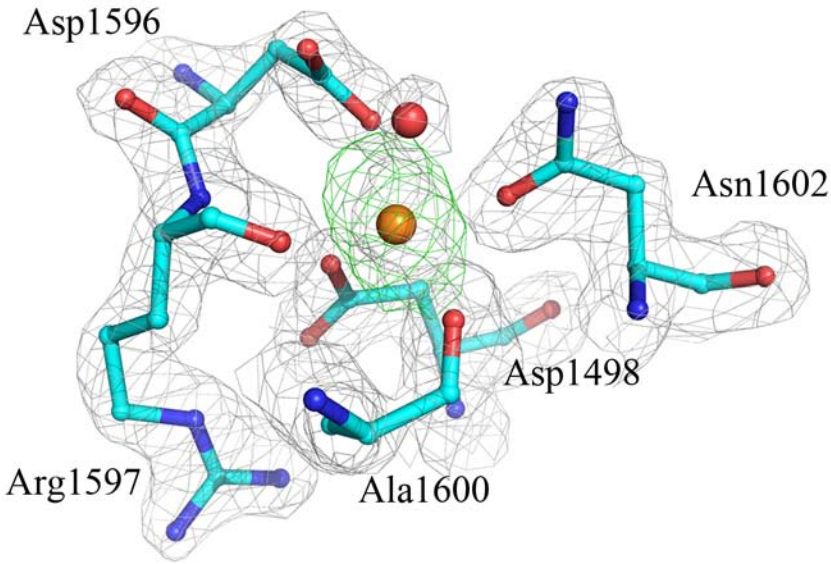
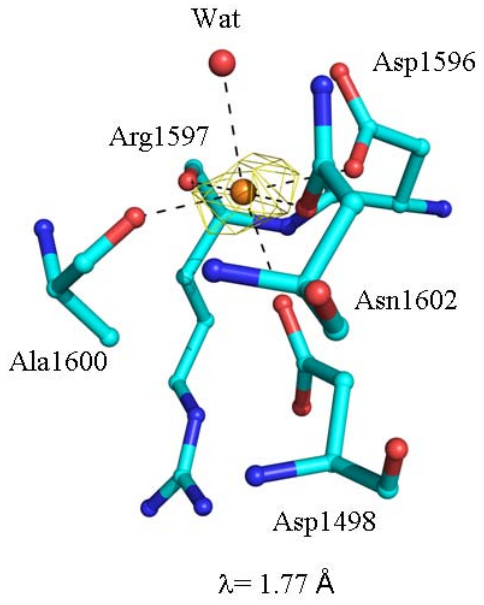

Asp1596
Asn1602
Asp1498
Arg1597
Ala1600

Figure S2

A


Wat
Asp1596
Arg1597
Asn1602
Ala1600
Asp1498
λ= 1.77 Å

B

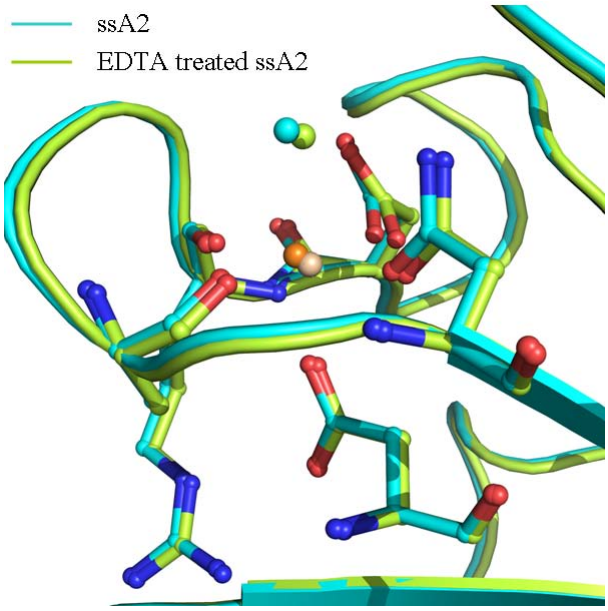

ssA2
EDTA treated ssA2

Figure S3

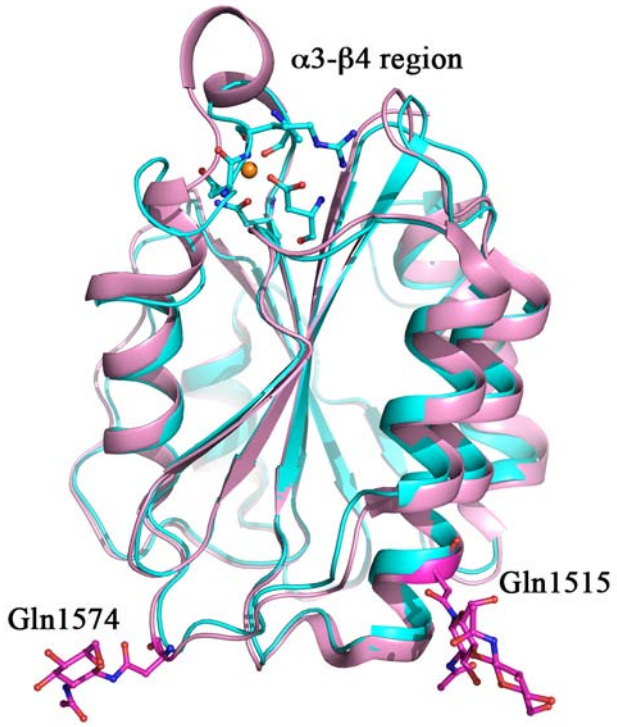
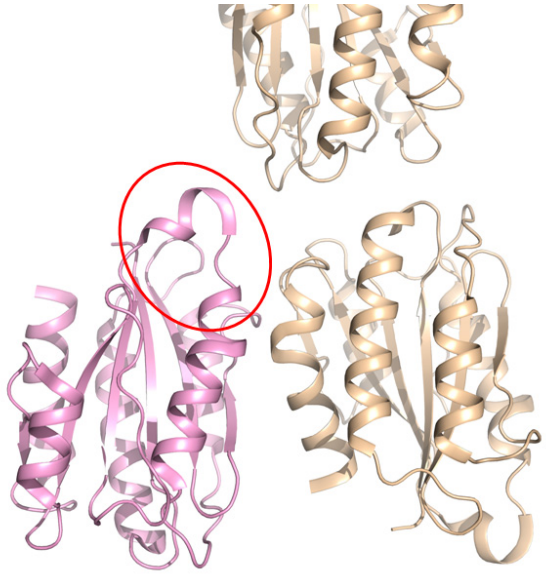


Figure S4

A



B

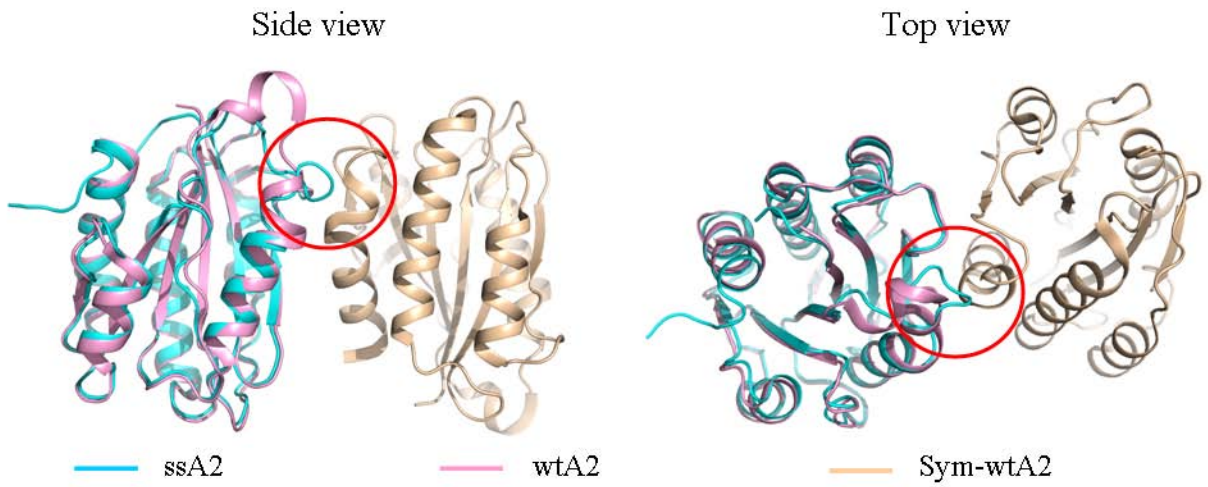


Figure S5