Supporting information

Structural insights and the surprisingly low mechanical stability of the Au-S bond in the gold-specific protein GolB

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Experimental Section.

Protein expression. The plasmid of *Salmonella typhimurium* golb gene is a generous gift from Prof. Chuan He at The University of Chicago. The gene encoding the protein GolB protein (GMQFHIDDMTCGGCASTVKKTILTLDANATVRTDPATRLVDVETSLSAEQIAAALQKAGFPPRER) was amplified from the plasmid by standard polymerase chain reaction (PCR) methods. The fusion protein 6×His-MBP-TEV-GolB was sub-cloned into a pET28a vector, and the resulting construct was subsequently transformed into BL21 (DE3) cells. The fusion protein was expressed in LB medium containing 50 μg/mL kanamycin after induction with 1 mM IPTG at 15℃. To obtain purified GolB, *E.coli* BL21 (DE3) cells containing the recombinant plasmid that had been cultured overnight were collected by centrifugation. The pellet was re-suspended in buffer (50 mM Tris-HCl pH 7.0, 500 mM NaCl and 5% v/v glycerol) and dissociated by microfluid. The supernatant was obtained by centrifuging the cell lysate at 20,000 rpm and 277 K for 1 h. Standard Ni-affinity chromatography (His-Trap FF) was performed for preliminary purification of the His-tagged fusion protein from the supernatant. The enrichment fusion protein was digested by TEV protease at 277 K overnight. Ni-affinity chromatography (His Trap HP) was used again to obtain GolB, which was separated from the 6×His-MBP-TEV fusion protein. High purity GolB was obtained after further purified by size-exclusion chromatography (Superdex 30) and was concentrated.

Crystallization. Crystal screening was performed at 293 K by the sitting-drop vapour-diffusion method. A 200 nanolitre protein solution (4.43 mM) was mixed with 200 nanolitre reservoir solution and equilibrated against 30 microlitre reservoir solution. Commercial crystallization kits from Hampton Research and Qiagen were used for crystal screening. Initial crystals of GolB were observed under the following condition: 2.0 M ammonium citrate tribasic pH 7.0, 0.1 M BIS-TRIS propane pH 7.0. Single crystals were obtained by further optimization of salt concentration and pH values. For heavy atom derivative crystals preparation, we added 10 mM $KAu(CN)_2$ to a cryo-protection solution (2.0 M ammonium citrate tribasic pH 7.0, 0.1

M BIS-TRIS propane pH 7.0, 25% glycerol), soaked the crystals for about 4 hoursand then the data were collected at home source diffraction system.

Single-molecule AFM. First, the gene encoding the protein GolB with a β-hairpin fragment (15 aa EWTYDDATKTFIVTE) from GB1 inserted in the C10XXC13 metal ion binding loop was amplified by PCR methods using the primers (GolB-beta-F: CGGGATCCATGCAGTTCCATATTGATGACATGACCTGCGGCGAATGGACCTACGACGACGCTACCAAAACCTTCACGGTTA CCGAAGGCTGCGCCAGTACGGTAAAAAAGA and GolB-R: GGGGTACCTTACTAAGATCTCCTCTCGCGCGGCGGGAAAC). Then the gene encoding the (beta GB1-GolB)4 hybrid polyprotein was constructed using standard molecular biology techniques to splice GB1 and GolB with β-hairpin in an iterative approach[1]. (beta GB1–GolB)4 was expressed in BL21 and purified by Ni^{2+}-affinity chromatography. Single-molecule AFM experiments were performed on a commercial AFM (NanoWizard II, JPK Instruments, Berlin, Germany). In the force spectroscopy experiments, we first deposited 30 μL of the protein at a concentration of around 0.3 mg/mL on a glass substrate for 5 min. Next, we filled in the fluid chamber with 1.5 mL Tris-HCl buffer (pH 7.4). Based on equipartition theorem, the cantilever (MLCT, BRUKER, USA) with a spring constant of ~40 pN/nm was then calibrated after equilibrating in the solution for around 30 min. In a typical experiment, the cantilever was brought into contact with the surface at a constant speed of 1000 nm/s. Then the cantilever stayed on the surface with a contact force of around 9 nN for 1.5 s to to ensure the adhesion between protein and the tip. Subsequently, the cantilever was retracted to stretch the protein molecule being picked up from the surface. The force curves were recorded automatically by the software from JPK and then analyzed by a home-written protocol in Igor 6.37 (WaveMetrics, Lake Oswego, OR). At least 200 events were recorded of each protein and the force distributions were then fitted by Gaussian distribution.

UV-visible measurement of metal binding experiment. The (beta GB1-GolB)4 and (GB1-GolB)4 polyprotein were reduced with 1 mM DTT overnight at 4 °C and passed through 10 DG desalting column using 100 mM NaCl, 100 mM Tris-HCl, pH 7.5. About 1 μM of GolB protein was used immediately for gold (I) binding experiment in the same buffer. For all measurements, baseline was recorded with protein itself and UV spectra differences were recorded for each measurement. Gold(I) binding was monitored as an increase in absorption band at 250-255 nm[2,3]. Concentration and absorbance were corrected for final volume.

Circular dichroism experiments. (Beta GB1-GolB)$_4$ and (GB1-GolB)$_4$'s (around 0.04mg/mL) CD spectra in 1×PB was obtained by JASCO J-815 CD spectropolarimeter (JASCO, Japan). The experiment was done in a quartz cell with 0.1mm path length from 250nm to 190nm wavelength at the room temperature.

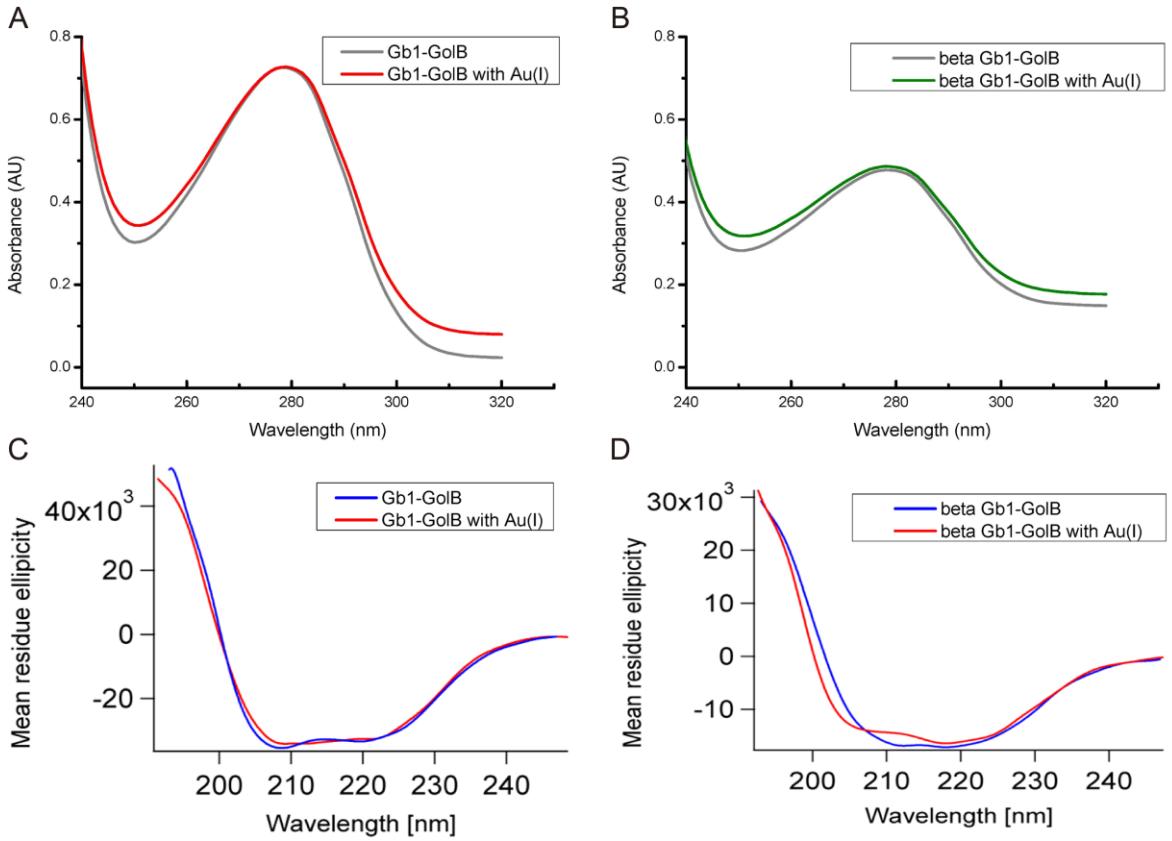

Figure S1 UV-vis spectra of $(GB1\text{-}GolB)_4$ protein loaded with Au(I) (A), (beta $GB1\text{-}GolB)_4$ protein loaded with Au(I) (B), CD spectra of $(GB1\text{-}GolB)_4$ protein loaded with Au(I) (C) and (beta $GB1\text{-}GolB)_4$ protein loaded with Au(I) (D).

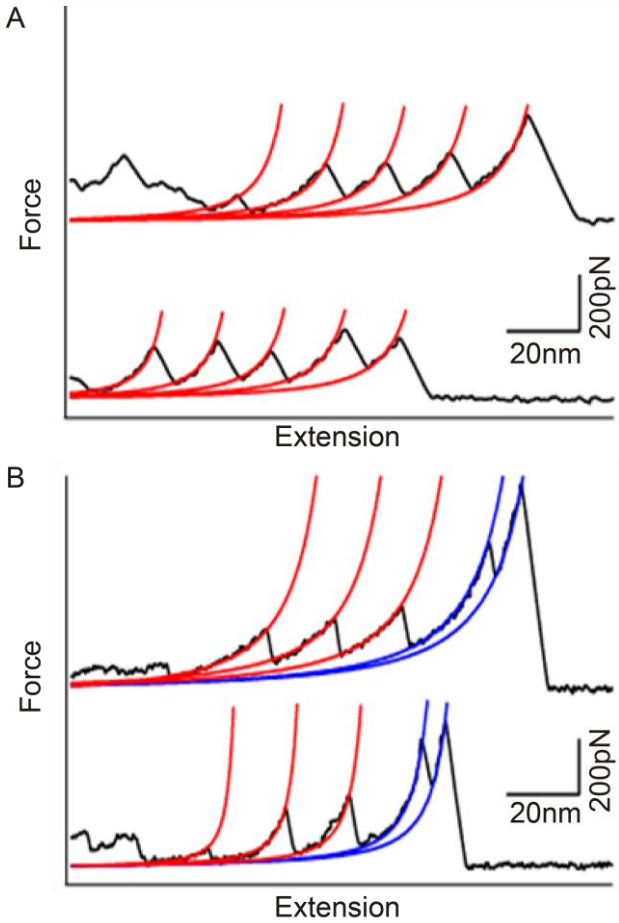

Figure S2 Representative curves for stretching (GB1-GolB)4 (A) and (beta GB1-GolB)4 under oxidation conditions (B). Cys^{10} and Cys^{13} of GolB form intramolecular disulphide bonds in the polyprotein. Clearly, no unfolding events of GolB can be detected in both proteins, indicating that GolB is mechanically labile and unfolds at forces below the force detection limit of our AFM setup (~ 10 pN). Because the loop trapped by Cys^{10} and Cys^{13} in wild type GolB is too short, we can only resolve the rupture events of disulphide bonds in GolB with loop insertion (~ 9 nm) in some cases (highlighted in blue). However, because the rupture forces for disulfide bonds are much higher than the nonspecific interaction forces used to attach the polyprotein to the cantilever and the substrate, the number of events corresponding to rupture disulfide bonds are much less than the number of events for GB1 unfolding. Clearly, the rupture forces for disulfide bonds are much higher than the rupture forces for Au(I)-S or Cu(I)-S bonds in holo GolB.

Table S1 Crystallization Method

Method	Sitting-drop vapour-diffusion
Plate type	Corning 3552
Temperature (K)	293
Protein concentration	31 mg/mL
Buffer composition of protein solution	50 mM Tris-HCl pH 7.5, 200 mM NaCl.
Composition of reservoir solution	2.0 M Ammonium citrate tribasic pH 7.0, 0.1 M BIS-TRIS propane pH 7.0.
Volume and ratio of drop	200 nL protein/200 nL reservoir
Volume of reservoir	microlitre.

Table S2 Data collection and processing

We finished all of the data collection works by use F-RE++ and R-AXIS Ⅳ of RIGAKU.

	Native	GolB(Au+ soak)	GolB(TCEP soak)	GolB(TCEP and Au+ soak)
Diffraction source	RIGAKU F-RE++	RIGAKU F-RE++	RIGAKU F-RE++	RIGAKU F-RE++
Wavelength (Å)	1.54	1.54	1.54	1.54
Temperature (K)	100	100	100	100
Detector	R-AXIS IV	R-AXIS IV	R-AXIS IV	R-AXIS IV
Crystal-detector distance (mm)	90	90	130	140
Rotation range per image (°)	0.5	0.5	0.5	0.5
Total rotation range (°)	180	180	90	120
Exposure time per image (s)	360	300	300	300
Space group	P65 22	P65 22	P65 22	P65 22
a, b, c (Å)	36.993, 36.993, 169.761	37.462, 37.462, 167.976	37.007, 37.007, 169.932	37.093, 37.093, 169.911
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120
Mosaicity (°)				
Resolution range (Å)	50.00-1.40	50.00-1.41	50.00-1.80	50.00-2.00
Total No. of reflections	221204	199923	51000	66890
No. of unique reflections	22949	23974	7012	5297
Completeness (%)	99.1 (93.1)	93.9 (42.3)	97.8 (96.9)	99.5 (100)
Redundancy	9.64 (3.04)	8.43 (1.56)	7.3 (5.0)	12.6 (9.1)
⟨ I/σ(I)⟩	20.25 (1.78)	19.05 (2.26)	34.28 (1.38)	52.94 (20.35)
Rr.i.m.	5.0% (62.7%)	6.9% (48.8%)	4.5% (9.6%)	7.0% (12.0%)
Overall B factor from Wilson plot (Å2)			18.061	17.090

† Only the redundancy-independent merging R factor $R_{r.i.m.}$ or R_{meas} should be reported. If these values are not available, they may be estimated by multiplying the conventional R_{merge} value by the factor $[N/(N-1)]1/2$, where N is the data multiplicity [the $R_{r.i.m}$ are estimated from R_{merge} value as suggested.].

‡ State here if there are any anomalies in the Wilson plot, such as spikes arising from ice rings, etc.

Table S3 Statistics of Refinement

	Native	GolB (TCEP and Au+ soak)
R factor‡ (%)	16.6	20.8
Rfree‡ (%)	19.1	23.9
R.m.s. deviation from ideal geometry		
Bonds (Å)	0.007	0.01
Angles (°)	1.256	0.90
Rms ChirVolume	0.064	0.031
Non-crystallography contents		
Molecules	1	1
Redisues	65	62
Waters	61	35
Au	-	2
Ramachandran		
Preferred regions	96.88%	97.77%
Allowed regions	3.12%	3.23%
outliers	0	0

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