**Supporting Information**

**for**

# Crystal structure of a DNA duplex cross-linked by 6-thioguanin–6-thioguanin disulfides: reversible formation and cleavage catalyzed by Cu(II) ion and glutathione

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## MATELIALS AND METHODS

DNA dodecamer with a sequence d(CGCGAXXBCGCG) (**ODN-I** hereafter) where X and B residues are 2'-deoxy-6-thioguanosine and 2'-deoxy-5-bromouridine, respectively, were synthesized by a DNA synthesizer and then purified by HPLC and reversed-phase chromatography. A monomer unit for 2'-deoxy-6-thioguanosine residue was purchasted from Glen Research corp., and used according to an instraction of the manufacture.

Prior to crystallization, 2 mM DNA was mixed with 2 mM copper(II) chloride at room temperature. Crystallizations were performed by the hanging-drop vapor diffusion method. Single crystals were obtained in a droplet prepared by merging 1 μl of DNA/copper(II) mixed solution mentioned above and 1 μl of crystallization solution containing 50 mM MOPS (pH 7), 10 mM spermine, 250 mM ammonium nitrate and 10% 2-methyl-2,4-pentanediol, which was equilibrated against 250 μl of 40% 2-methyl-2,4-pentanediol.

A single crystal was scooped from a droplet by a cryo-loop (Hampton Research) and then frozen immediately in liquid nitrogen. An X-Ray diffraction dataset was collected at structural biology beamline BL-17A in Photon Factory (Tsukuba, Japan). The X-ray data was processed by the program *XDS* (Kabsch 2010). The initial phase was determined by the Single-wavelength Anomalous Dispersion (SAD) method using the program *AutoSol* in the *Phenix suite* (Adams *et al*., 2010; Grosse-Kunstleve *et al*., 2003; Terwilliger *et al*., 2009). The molecular structure was constructed and manipulated with the program *Coot* (Emsley *et al*., 2004, 2010). The atomic parameters were refined with the program *phenix.refine* from the *Phenix* suite (Adams *et al*., 2010; Afonine *et al*., 2012) through a combination of simulated-annealing, crystallographic conjugate gradient minimization refinements and B-factor refinement. Molecular drawings of the structure were performed by the program *PyMOL* (DeLano 2008). The statistics of data collection and structure refinement are summarized in Table S1. The atomic coordinate and experimental data of the structure have been deposited in the Protein Data Bank (PDB) with the ID code 6YIQ.

HPLC was performed on a reverse phase silica gel column (7.6×250 mm) (Inertsil ODS-3; GL Science Inc., Japan) with a linear gradient of $CH_3CN$ in 0.1 M triethylammonium acetate buffer (pH 6.8) with a flow rate 1 mL/min.

**Table S1.** Crystal data and statistics of data collection and structure refinement

| | |
|---|---|
| Crystal data | |
| Space group | *I*222 |
| Unit cell (Å) | $a$ = 45.3, $b$ = 46.5, $c$ = 76.1 |
| No. of DNA strands in AU [a] | 2 |
| Data collection | |
| Beamline | BL-17A |
| Wavelength (Å) | 0.91942 |
| Resolution (Å) | 39.7 - 2.0 |
| of the outer shell (Å) | 2.1 - 2.0 |
| Unique reflections | 5619 |
| Completeness (%) | 99.8 |
| in the outer shell (%) | 98.8 |
| $R_{merge}$ [b] (%) | 4.4 |
| in the outer shell (%) | 28.7 |
| Redundancy | 6.2 |
| in the outer shell | 6.7 |
| $I/\sigma(I)$ | 19.2 |
| in the outer shell | 5.2 |
| Structure refinement | |
| Resolution range (Å) | 39.7 - 2.0 |
| Used reflections | 5598 |
| $R$-factor [c] (%) | 24.7 |
| $R_{free}$ [d] (%) | 28.2 |
| R.m.s.d. bond length (Å) | 0.013 |
| R.m.s.d. bond angles (°) | 1.4 |

[a] Number of nucleic acid strands in the asymmetric unit.

[b] $R_{merge} = 100 \times \Sigma_{hklj}|I_{hklj} - \langle I_{hklj}\rangle| / \Sigma_{hklj}\langle I_{hklj}\rangle$.

[c] $R$-factor $= 100 \times \Sigma||F_o| - |F_c|| / \Sigma|F_o|$, where $|F_o|$ and $|F_c|$ are optimally scaled observed and calculated structure factor amplitudes, respectively.

[d] Calculated using a random set containing 10% of observations.

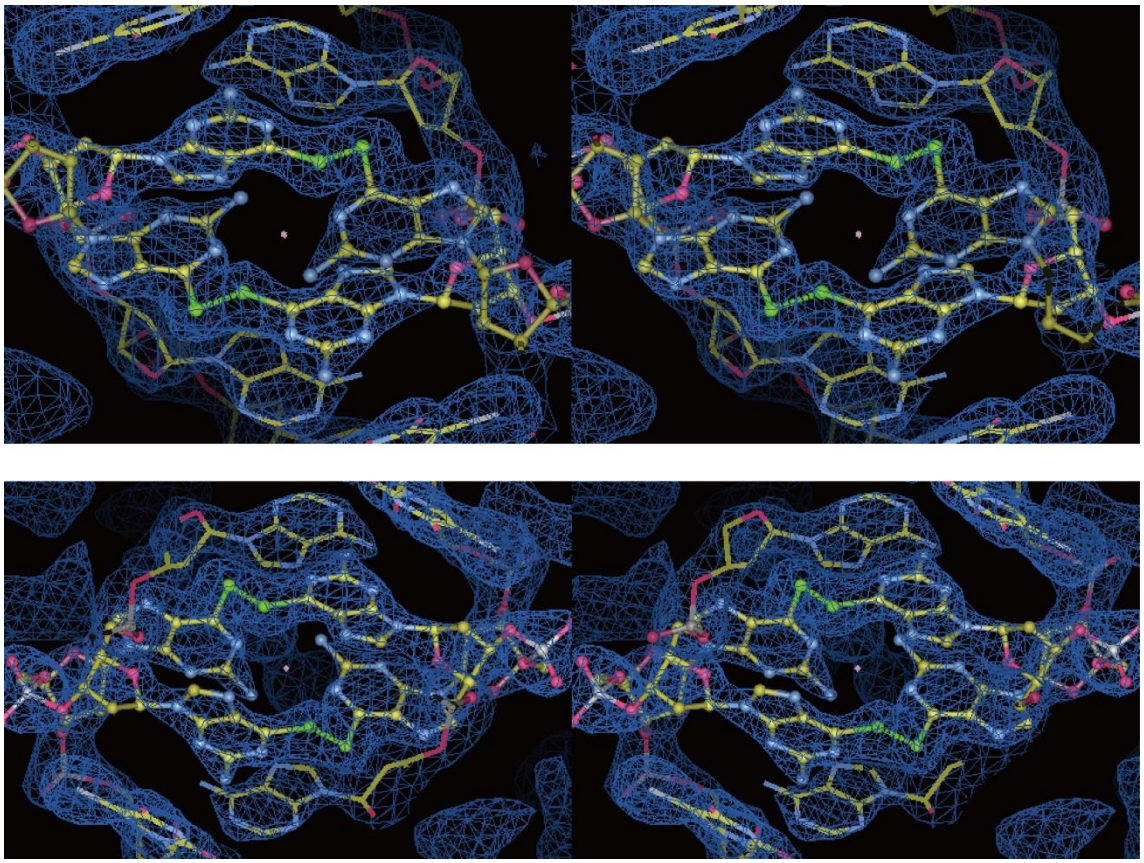

**Figure S1.** Disulfide-bonded base pairs between 6-thio-G residues with $2|F_o|-|F_c|$ map (blue mesh: 2σ contour level). 6-thio-G residues are illustrated in ball-and-stick model. Views are from the minor (above) and major (below) grooves of the two consecutive disulfide-bonded base pairs.

**Cleavage of the disulfide bonds by glutathione (GSH)**.

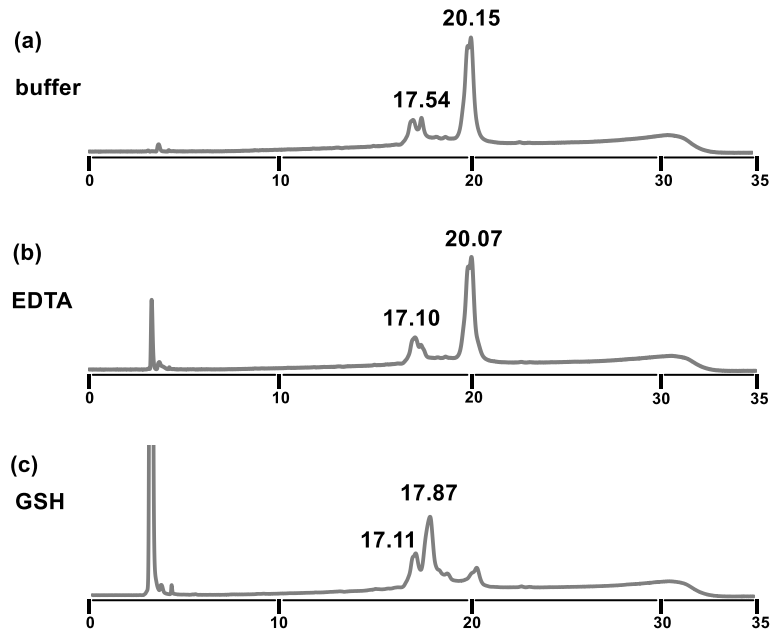


**Figure S2.** HPLC profiles of solutions in Fig. S3. A linear gradient of $CH_3CN$ from 7 % to 21% (20 min) to 28% (5 min) was used.

Experimental procedures are shown in Fig. S3. A solution containing **duplex-I'** and Cu(II) ions were kept at room temperature for overnight. Formation of **duplex-I'ss** was confirmed by HPLC (Fig. S2a). The solution was separated and appropriately diluted to give solutions containing EDTA, or GSH. Solutions were kept at room temperature overnight and analyzed by HPLC (Fig. S2b, c).

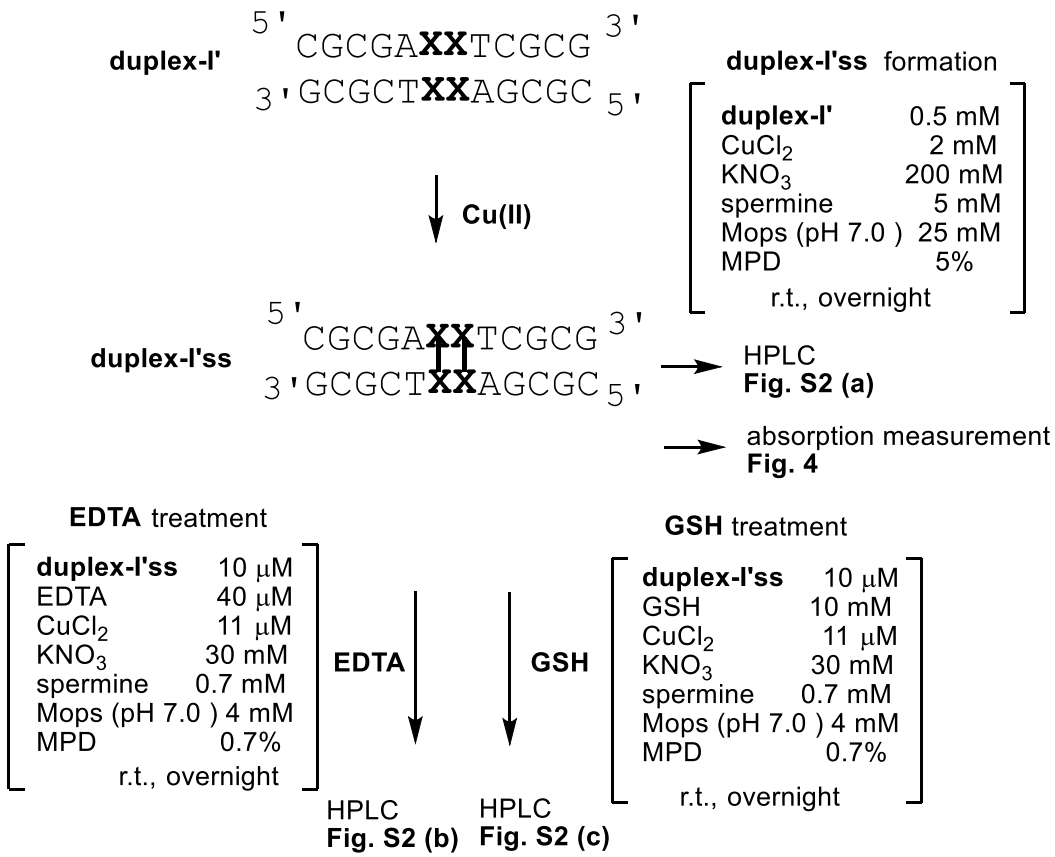


**Figure S3**. A schematic representation for experiments. In order to prepare **duplex-I'ss** for HPLC analyses and absorption measurement (Fig. 4), the amount of **duplex-I'** was larger than that in Fig. 3b.

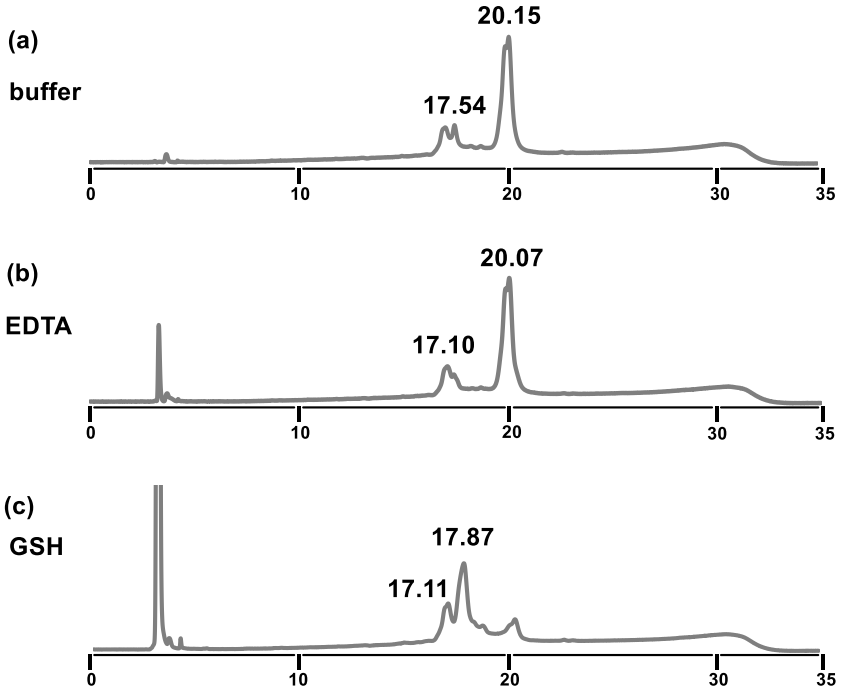


**Figure S3.** HPLC profiles of solutions in Fig. S2. A linear gradient of $CH_3CN$ from 7 % to 21% (20 min) to 28% (5 min) was used.

**Effect of $KBrO_3$ for disulfide bonds formation**.

In to solutions containing **duplex-I'** were added Cu(II) ions or $KBrO_3$., and solutions were kept at roomtemperature for 24h.

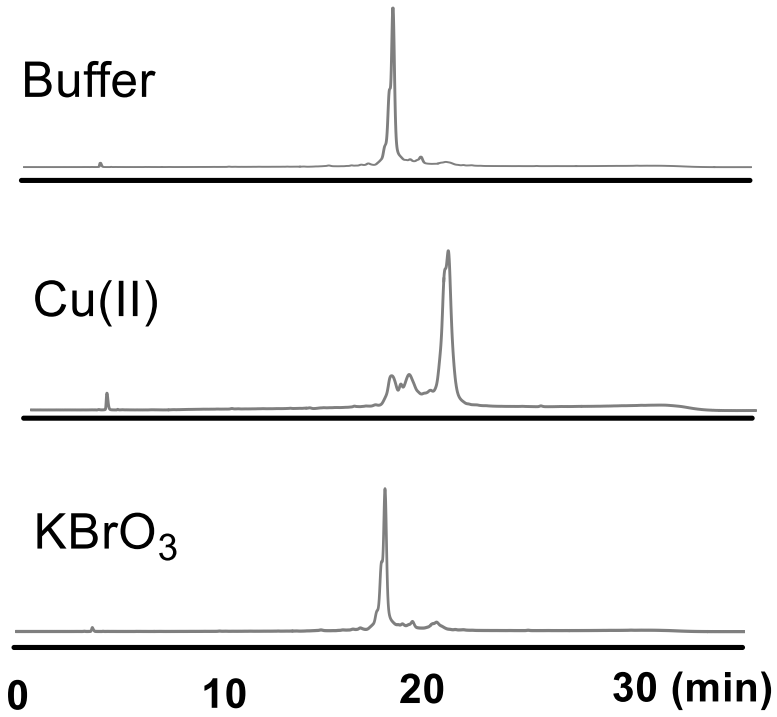


**Figure S4.** HPLC profiles of solutions.

(a) **duplex-I'** 1 mM
$KNO_3$ 400 mM
Mops (pH 7.0 ) 50 mM

(b) **duplex-I'** 1 mM
$CuCl_2$ 2 mM
$KNO_3$ 400 mM
Mops (pH 7.0 ) 50 mM

(c) **duplex-I'** 1 mM
$KBrO_3$ 2 mM
$KNO_3$ 400 mM
Mops (pH 7.0 ) 50 mM

A linear gradient of $CH_3CN$ from 7 % to 21% (20 min) to 28% (5 min) was used.

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