Supporting Information: Protein Binding has a Large Effect on Radical Mediated DNA Damage.

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Contents:

General Methods. Unless otherwise specified, chemicals were purchased from Aldrich or Fisher Scientific and enzymes were obtained from New England Biolabs. Hbb was isolated as previously described.[1] Oligonucleotides were synthesized via standard automated DNA synthesis techniques using an Applied Biosystems model 394 instrument. The Pac-dA and ^{i}Pr-Pac-dG phosphoramidites were employed for the synthesis of oligonucleotides containing **3** and **4**. Pivaloyl anhydride/2,6-lutidine/THF (1:1:8) was used as capping reagent and 1 M t-butyl-hydroperoxide in toluene was used as oxidizing

reagent.[2] The oxidation time is 40 s and the capping time is 25 s. Deprotection of the nucleobases and phosphate moieties as well as cleavage of the linker were carried out under mild deprotection conditions (28% aq. NH_3, room temperature, 3 h). Oligonucleotides were purified by 20% denaturing polyacrylamide gel electrophoresis and characterized by ESI-MS. Oligonucleotides containing **3** or **4** were subjected to additional purification by reversed-phase HPLC on a RP-C18 column (VARIAN, Microsorb-MV 100-5 C18 250 × 4.6 mm). Monitoring was carried out at 260 nm. The peak of interest was collected using the following gradient conditions: 0-5 min 100% A, 5-20 min 0-15% B in A, 20-55 min 15-22% B in A, 55-60 min 22-80% B in A, 60-65 min 80-0% B in A, 65-75 min 100% A, at a flow rate 1.0 mL/min) [A: 0.05 M (Et_3NH)OAc (pH 7.0)/MeCN 95:5; B: 0.05 M (Et_3NH)OAc (pH 7.0)/MeCN 50:50]. Radiolabeling was carried out according to the standard protocols.[3] [γ-^{32}P]ATP was purchased from Amersham Pharmacia Biotech. Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics Phosphorimager equipped with ImageQuant Version 5.1 software. ESI-MS spectra were collected using a Thermoquest LCQ-Deca Ion Trap instrument.

Protein binding and cross-linking reactions.

All reactions involving Hbb were carried out in siliconized tubes. Hbb was isolated as previously described.[1] Protein dilutions were made in 25 mM HEPES/NaOH (pH 7.5), 50 mM NaCl, 10% glycerol and 50 μg mL^{-1} bovine serum albumin. The binding of DNA (20 nM, 5'-^{32}P-**5**-**8**) with Hbb (600 nM) was performed in 10 mM HEPES/NaOH (pH 7.5), 25 mM NaCl, 5 mM $MgCl_2$, 5% glycerol and 0.5 μg mL^{-1} non-specific duplex DNA (total volume 20 μL). The non-specific duplex DNA was the same as that used previously.[1] The binding is complete by incubation at r.t. for 15 min and 4 °C for 15 min. The cross-link reaction was carried out immediately after the binding reaction by UV-irradiation (350 nm) at r.t. for 2 h. Photolyses were carried out under aerobic conditions in a Rayonet photoreactor equipped with 16 lamps that emit at a maximum wavelength = 350 nm. The cross-linking reaction in the absence of Hbb was performed in the same conditions, including buffer, incubation, and UV-irradiation.

Competitive kinetics for ICL formation in the presence or absence of Hbb.

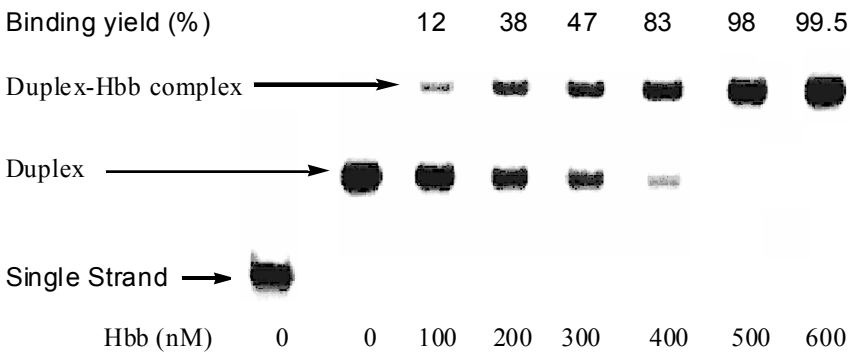
DNA samples (34 μL, 23.5 nM) were prepared in 10 mM HEPES/NaOH (pH 7.5), 25 mM NaCl, 5 mM $MgCl_2$, 5% glycerol, 0.5 μg mL^{-1} non-specific duplex DNA and different concentrations of 2-mercaptoethanol (BME) (0-4 mM). Each sample was divided into two portions. One portion was added 4 μM Hbb (3 μL) and another was added the same amount of protein dilution buffer (25 mM HEPES/NaOH (pH 7.5), 50 mM NaCl, 10% glycerol and 50 μg mL^{-1} bovine serum albumin). All samples (total volume 20 μL, 20 nM) were incubated at r.t. for 15 min and 4 °C for 15 min, irradiated with 350 nm UV for 2 h, immediately quenched with 20 μL loading buffer (95% formamide, 10 mM EDTA, and 20 μM non-specific DNA), heated at 90 °C for 3 min, and stored at -80 °C until subjecting to PAGE analysis. The cross-linked reactions (6 μL) were loaded onto 12% polyacrylamide (19:1) in 0.5 × TBE buffer and electrophoresed at ~ 8 V cm^{-1} for 4 h at r.t.

Fe·EDTA reaction.

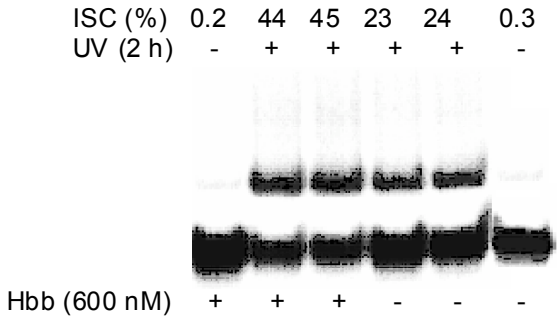
The cross-linked DNAs formed in duplexes **5** and **7** in the presence of Hbb were purified by 20% denaturing PAGE. The band containing cross-linked product was cut, crushed, and eluted with 200 mM NaCl, 20 mM EDTA (2.0 mL). The crude product was desalted using a C_{18}-SepPak column, eluting with H_2O (3 × 10.0 mL) followed by 1.0 mL MeOH:H_2O (3:2, 1.0 mL). Fe (II) · EDTA cleavage reactions were carried out in a buffer containing 10 mM sodium phosphate (pH 7.2), 10 mM NaCl, 100 μM EDTA, 1.0 mM sodium ascorbate, 2.0 mM H_2O_2, and 50 μM $(NH_4)_2Fe(SO_4)_2$ (total volume 20 μL) at 25 °C for 3 min, and quenched with 10 μL thiourea (100 mM). Samples were lyophilized, treated with 1.0 M piperidine (20 μL) at 90 °C for 20 min, lyophilized, dissolved in 10 μL H_2O: 95% formamide loading buffer (1:1), and subjected to electrophoresis on a 20% denaturing PAGE.

References

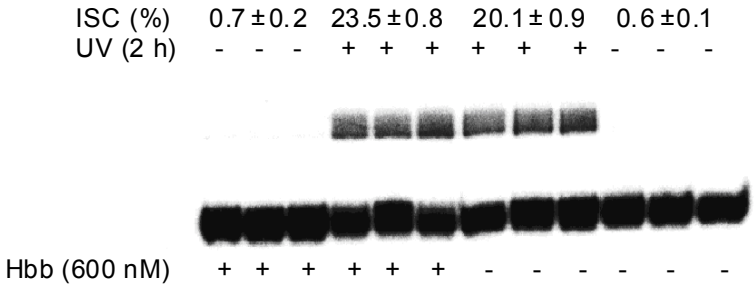
(1) Mouw, K. W.; Rice, P. A. *Mol. Microbiol.* **2007**, *63*, 1319-1330.
(2) Zhu, Q.; Delaney, M. O.; Greenberg, M. M. *Bioorg. & Med. Chem. Lett.* **2001**, *11*, 1105-1108.
(3) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning*; Cold Spring Harbor Laboratory, Cold Spring Harbor, NY., 1982.



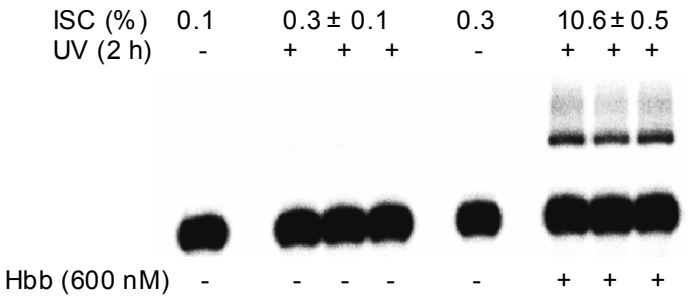
Supporting Information Figure 1. Phosphorimage autoradiogram of 12% non-denaturing PAGE analysis of Hbb-DNA binding (5'-^{32}P-**5)** with 20 nM DNA and different concentrations of Hbb.



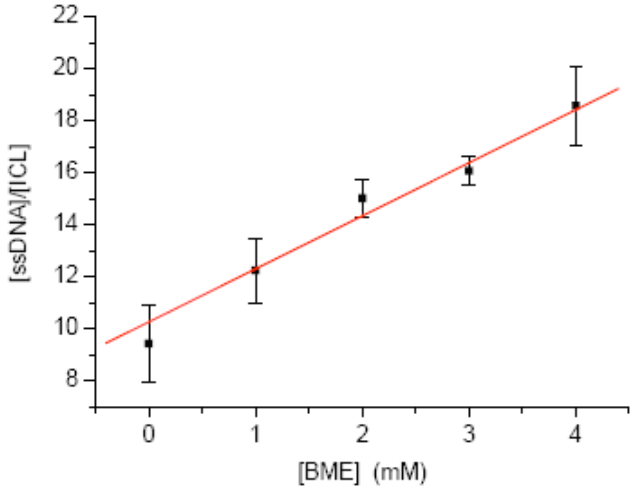
Supporting Information Figure 2. Phosphorimage autoradiogram of 12% denaturing PAGE analysis of ICL formation from **3** in 5'-^{32}P-**5** (strand containing **3** labeled) in the presence or absence of Hbb.



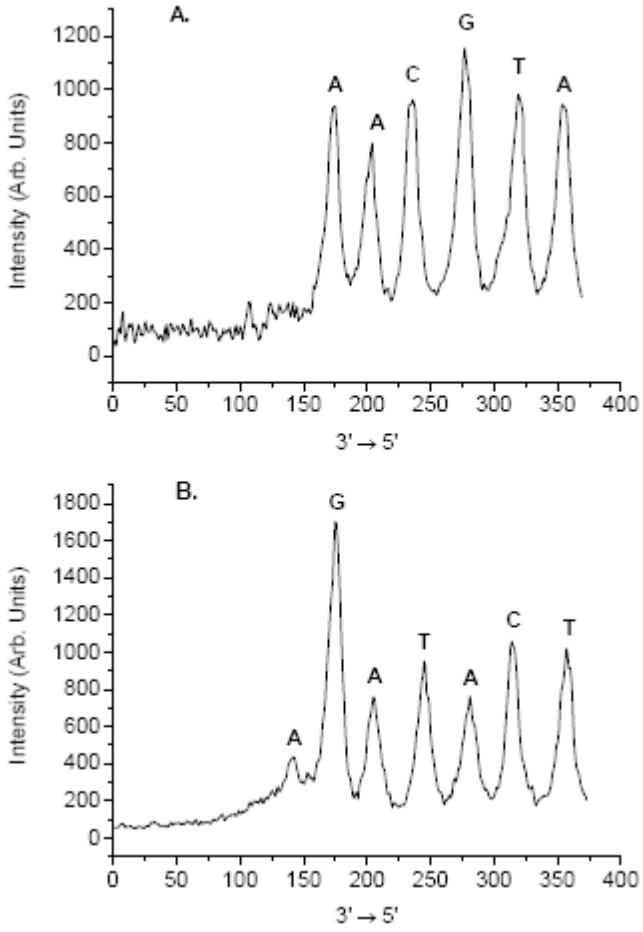
Supporting Information Figure 3. Phosphorimage autoradiogram of 12% denaturing PAGE analysis of ICL formation from **3** in 5'-^{32}P-**6** (strand containing **3** labeled) in the presence or absence of Hbb.



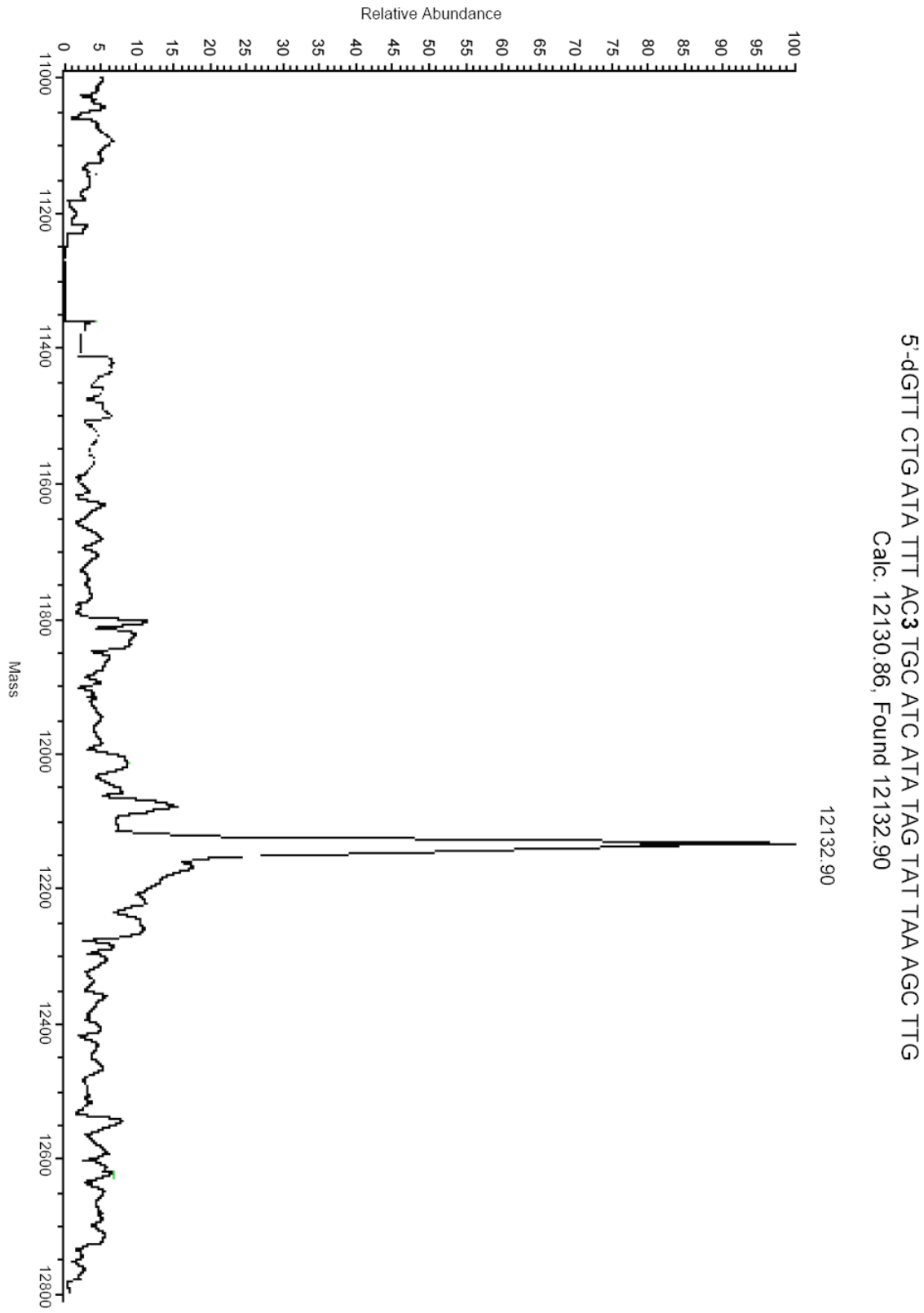
Supporting Information Figure 4. Phosphorimage autoradiogram of 12% denaturing PAGE analysis of ISC formation from **4** (strand containing **4** labeled) in 5'-^{32}P-**7** in the presence or absence of Hbb.



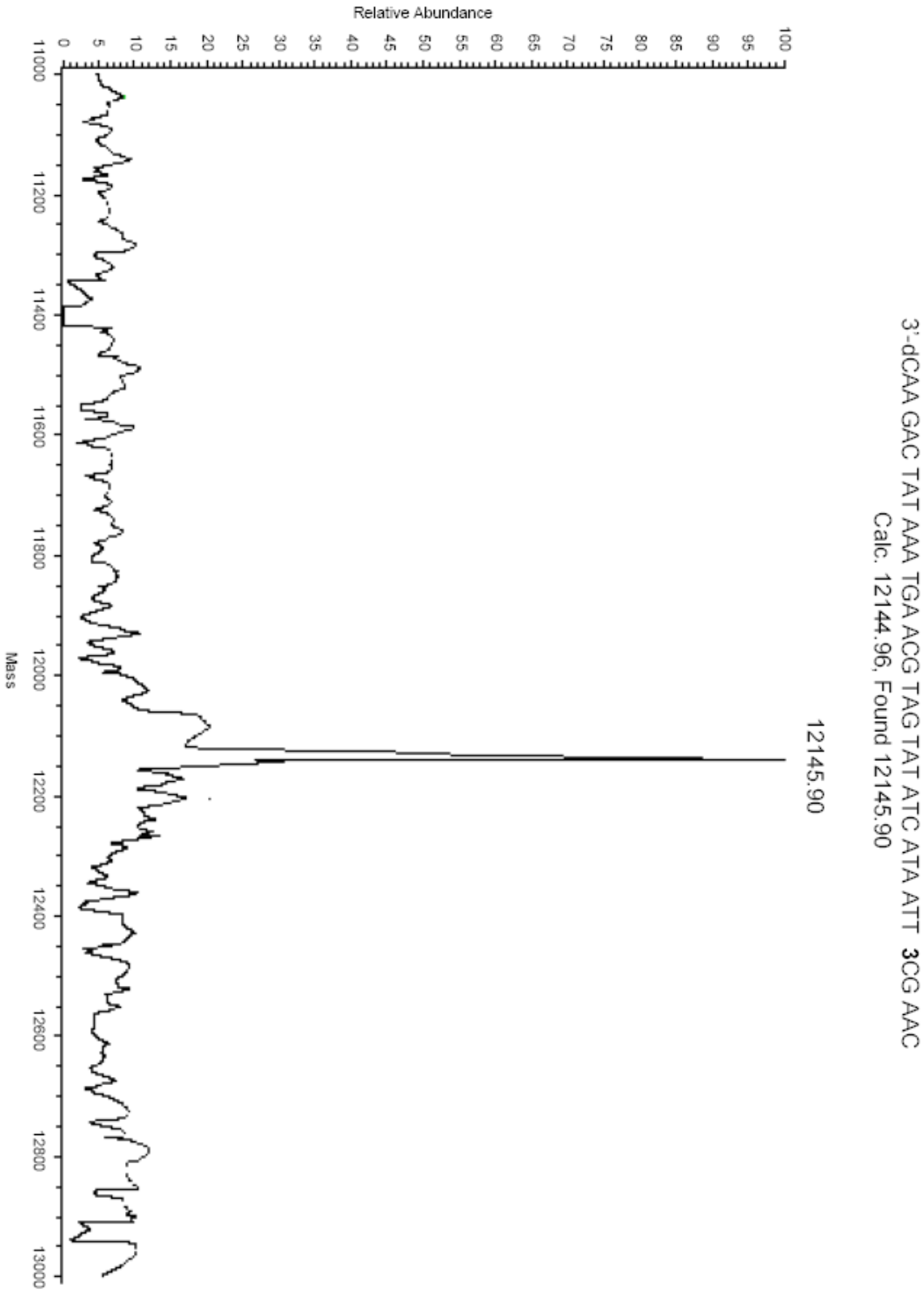
Supporting Information Figure 5. Ratio of [ssDNA]:[ICL] produced from photolysis of 5'-^{32}P-**7** (strand containing **4** labeled) in the presence of Hbb as a function of BME concentration (0 - 4 mM).



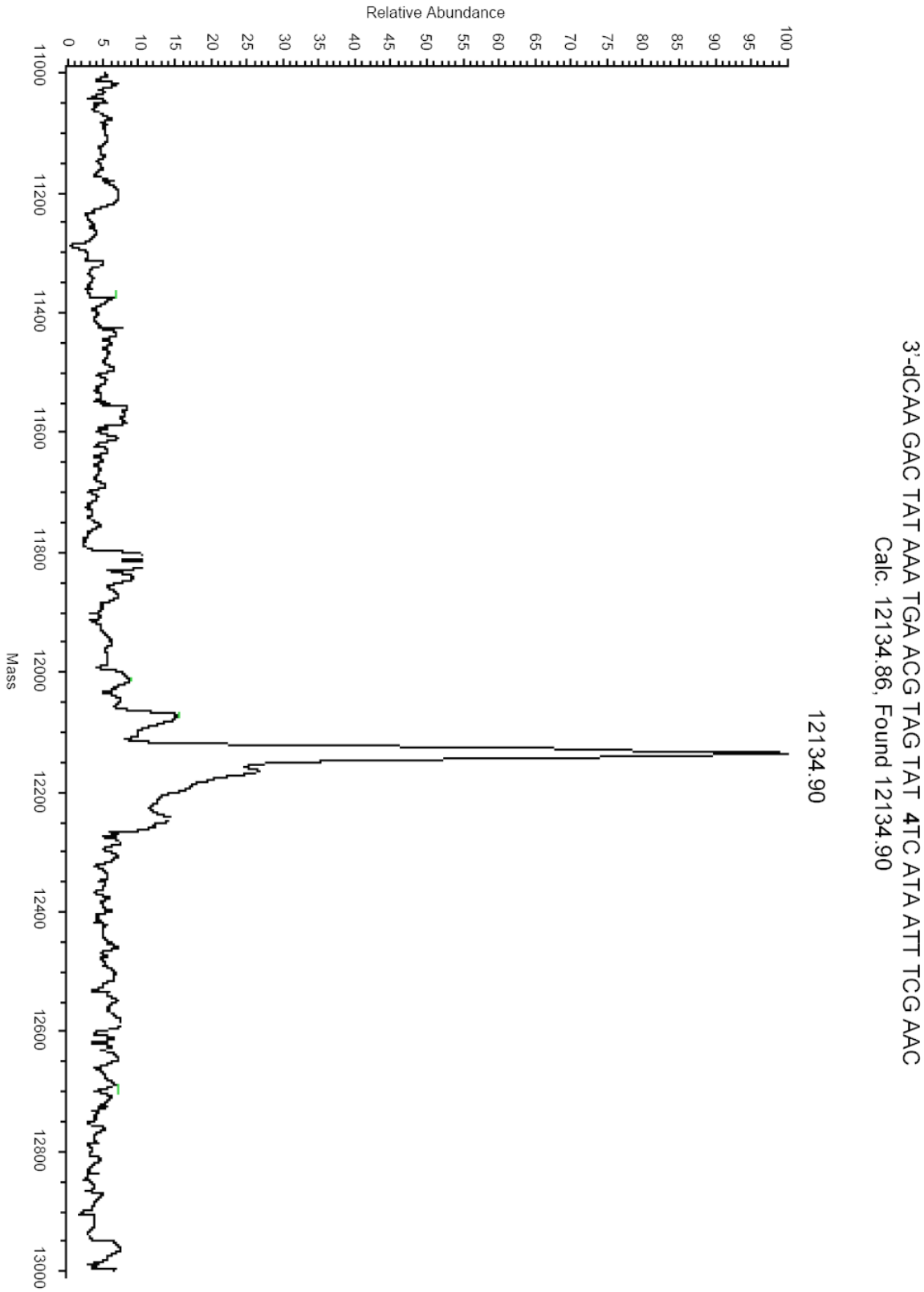
Supporting Information Figure 6. Histogram showing Fe·EDTA cleavage of ICL formed from **3** in 5'-^{32}P-**5** (**A**) and from **4** in 5'-^{32}P-**7** (**B**) generated by UV-irradiation (Complementary strand was labeled).



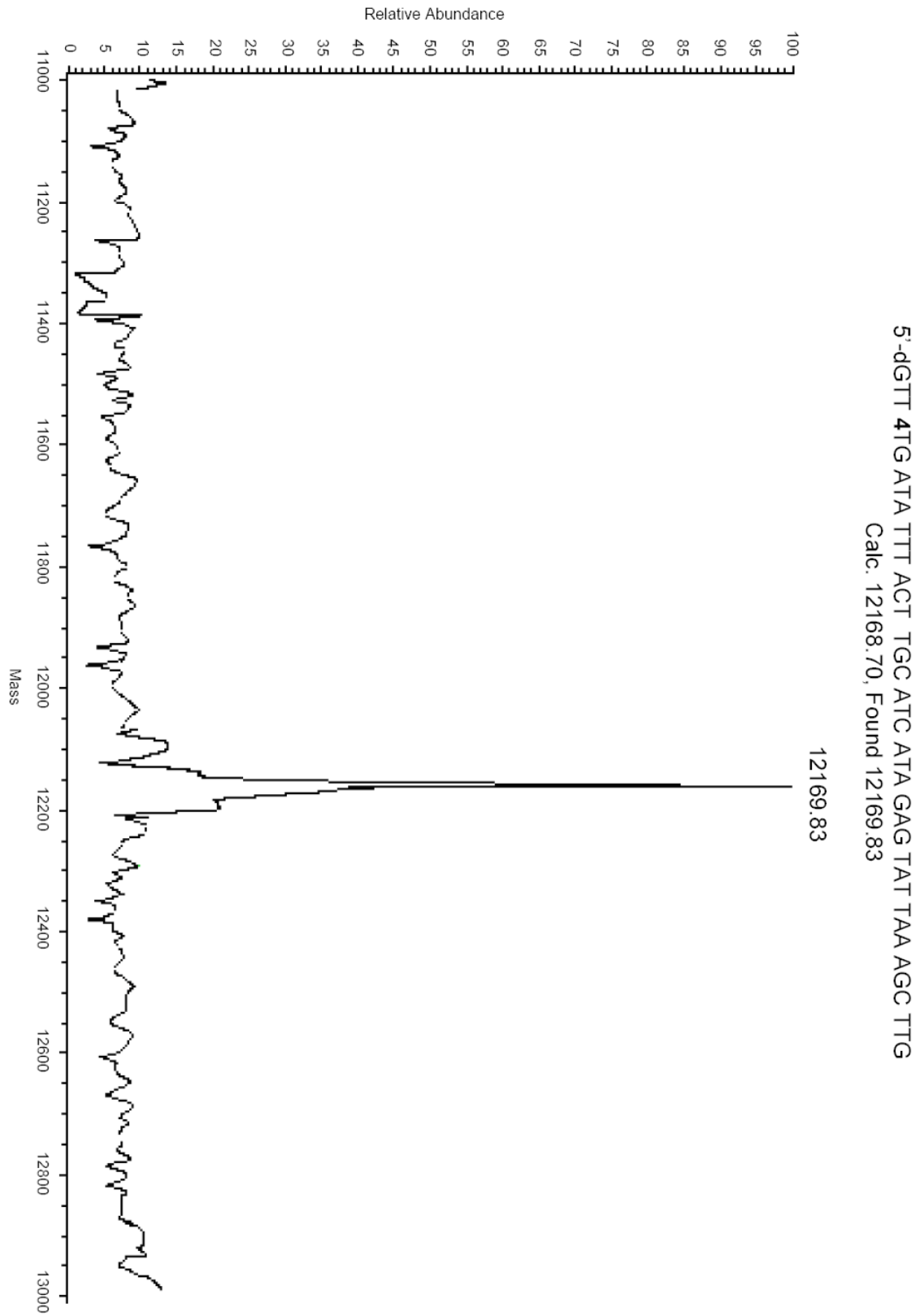
Supporting Information Figure 7. ESI-MS of the oligonucleotide containing **3** at Hbb bending site.



Supporting Information Figure 8. ESI-MS of the oligonucleotide containing **3** at non-bending site.



Supporting Information Figure 9. ESI-MS of the oligonucleotide containing **4** at Hbb bending site.



Supporting Information Figure 10. ESI-MS of the oligonucleotide containing **4** at non-bending site.