## **Supplementary Data for**

# A novel detection technique of polyamide binding sites by photo-induced electron transfer in <sup>Br</sup>U substituted DNA

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## MATERIAL AND METHODS

#### General

Reagents and solvents used in this study were purchased from standard suppliers and were used without further purification. Abbreviations used here: P, N-methylpyrrole; I, Nmethylimidazole; Fmoc, 9-fluorenylmethoxycarbonyl; HCTU, 1-[bis(dimethylamino)methylene]-5-chloro-1H-benzothiazolium 3-oxide hexafluorophosphate; DMSO, dimethylsulfoxide; DMF, dimethylformamide; NMP, 1-Methyl-2-pyrrolidone; DIEA, N,N-diisopropylethylamine;  $\beta$ -alanine; γ-aminobutyric acid; and Dp, N, N-dimethylaminopropylamine. <sup>1</sup>H NMR spectra were recorded on a JEOL JNM-FX 600 model NMR spectrometer. HPLC analysis was performed with a 4.6 x 150 mm column on a JASCO PU-2089 plus pump model with UV-2075 plus HPLC UV/VIS detector and a Chemcobond 5-ODS-H 10 x 150 mm column (Chemco Scientific Co., Ltd, Osaka, Japan) was used for the purification of polyamide conjugates. Electrospray lonization Time-of-Flight mass (ESI-TOF-MS) was recorded on BioTOF II ESI-TOF Bruker Daltonics Mass Spectrometer (Bremen, Germany). Flash column system was performed using Combi Flash Companion model (Teledyne Isco Inc., NE, USA). All primers including 5'-Texas Red-labeled primers were purchased from Sigma Aldrich and JBioS. A Thermo Sequence core sequencing kit was purchased from GE Healthcare. Polymerase chain reaction (PCR) was performed in iCyclear (BioRad). Hitachi 5500-S DNA sequencer machine was used for PAGE analysis and data was analysed by FLAGLYS version 2 software (Hitachi).

## **Polyamide Synthesis**

Polyamides, **1-4**, were synthesized by previous method (18). A  $\beta$ -ala-Wang resin was used in each case and programme based solid phase peptide synthesizer PSSM-8 with Fmoc chemistry have been used for repetitive coupling. Once the machine assisted synthesis finished, polyamides were detached from the resin by using Dp at 55 °C for 3 h. It was then freshly washed with cold Et<sub>2</sub>O and without further purification it was coupled with a pyrene butyric acid *N*-hydroxysuccinimide ester to achieve the final products. Within 1 h the reaction was finished and final purification was done by HPLC. The product was confirmed by TOF-MS and TOF-Mass.

Polyamide 1: ESI-TOF Mass m/z calcd. C<sub>84</sub>H<sub>98</sub>N<sub>24</sub>O<sub>13</sub> [M+2H]<sup>2+</sup> 825.39; found 825.26

Polyamide **2:** ESI-TOF Mass m/z calcd.  $C_{84}H_{98}N_{24}O_{13}$  [M+2H]<sup>2+</sup> 825.39; found 825.25. <sup>1</sup>HNMR for polyamide **1** and **2** was reported in the reference 2.

Polyamide 3: ESI-TOF Mass m/z calcd. C<sub>84</sub>H<sub>98</sub>N<sub>24</sub>O<sub>13</sub> [M+2H]<sup>2+</sup> 825.39; found 826.35

<sup>1</sup>H NMR (600 MHz, DMSO-d6)  $\delta$  10.227 (s, 1H; NH), 10.187 (s, 1H; NH), 10.985 (s, 1H; NH), 9.906 (s, 1H; NH), 9.867 (s, 1H; NH), 9.858 (s, 1H; NH), 9.828 (s, 1H; NH), 9.814 (s, 1H; NH), 8.339 (d, 1H; CH, J = 4.5), 8.227 (s, 1H; CH), 8.213 (s, 1H; CH), 8.176 (s, 1H; CH), 8.160 (d, 1H; CH, J = 1.8), 8.085 (d, 2H; CH, J=1.5), 8.021–7.996 (m, 4H, CH), 7.944 (m, 1H; CH), 7.89 (m, 1H; CH), 7.877 (m, 1H; CH), 7.505 (s, 1H; CH), 7.459 (s, 1H; CH), 7.288 (s, 1H; CH), 7.225 (s, 1H; CH), 7.213 (s, 1H; CH), 7.197 (s, 1H; CH), 7.133 (m, 2H; CH), 7.055 (s, 1H; CH), 7.027 (s, 1H; CH), 6.887 (br, 2H; NH), 6.846 (br, 3H; NH), 3.941 (s, 3H; N-CH<sub>3</sub>), 3.903 (s, 3H; N-CH<sub>3</sub>) 3.816 (s, 6H; N-CH<sub>3</sub>), 3.808 (s, 6H; N-CH<sub>3</sub>), 3.788 (s, 6H; N-CH<sub>3</sub>), 3.084 (m, 2H; CH), 2.973 (m, 4H; CH), 2.713 (d, 8H; CH, J = 2.7), 2.64 (s, 3H), 2.373-2.316 (m, 6H, CH) 2.174 (m, 6H) 1.961 (m, 2H; CH), 1.786-1.689 (m, 5H; CH), 1.138 (t, 3H; CH)

## Polyamide 4: ESI-TOF Mass m/z calcd. C<sub>84</sub>H<sub>98</sub>N<sub>24</sub>O<sub>13</sub> [M+2H]<sup>2+</sup> 825.39; found 825.25

<sup>1</sup>H NMR (600 MHz, DMSO-d6)  $\delta$  10.243 (s, 1H, NH), 10.219 (s, 1H, NH), 10.025 (s, 1H, NH), 10.005 (s, 1H, NH), 9.897 (d, 2H, NH), 9.885 (s, 1H, NH), 9.854 (s, 1H, NH), 8.370 (d, 1H; CH, J=4.2), 8.259 (s, 1H; CH), 8.247 (s, 1H; CH), 8.207 (d, 1H; CH, J=2.04), 8.199 (d, 1H; CH, J=2.1), 8.119 (d, 2H; CH, J=1.5), 8.026–8.055 (m, 4H, CH), 7.978 (m, 1H; CH), 7.922 (d, 1H; CH), 7.858 (m, 1H; CH), 7.540 (d, 2H; CH, J = 3), 7.272 (s, 2H; CH), 7.252 (s, 2H; CH), 7.178 (brs, 1H; CH), 7.162 (brs, 1H; CH), 7.149 (brs, 1H; CH), 6.931 (br, 3H; NH), 6.910 (br, 1H; NH), 6.879 (br, 1H; CN), 3.977 (d, 6H; N-CH<sub>3</sub>, J = 1.8), 3.851 (d, 6H; N-CH<sub>3</sub>, J = 1.2), 3.827 (d, 6H; N-CH<sub>3</sub>, J = 1.3), 3.802 (d, 6H; N-CH<sub>3</sub>, J = 1.6), 3.107 (m, 2H; CH), 3.010 (m, 2H; CH), 2.746 (d, 6H; CH, J = 2.4), 2.413-2.208 (m, 13H, CH) 1.994 (m, 2H; CH), 1.792-1.725 (m, 5H; CH), 1.186 (t, 3H; CH, J = 7.2)

## Preparation of TexasRed End-Labeled <sup>Br</sup>U substituted DNA

The DNA fragments were amplified by PCR using d<sup>Br</sup>UTP instead of dTTP using respective plasmids. The DNA1 was amplified by using forward 5'-TAATACGACTCACTATAGG-3' and reverse 5'-ATTTAGGTGACACTATAGAATAC-3' primers from pGEM3Z-601; and DNA2 was amplified by using forward 5'-GCAGGTCGACTCTAGAGGAT-3'and reverse 5'-GAGTCAGTGAGCGAGGAAG-3' primers from pUC18. Primers shown above either forward or reverse having TexasRed at the 5`-end were used for making either top strand or bottom strand labelled <sup>Br</sup>U-DNA. After PCR amplification, DNA was purified using the GenElute PCR Clean-Up Kit (Sigma-Aldrich, St. Louis, MO, USA) and quantified by Nanodrop.

## Photoreaction

Photoreaction sample was prepared by mixing 10 nM DNA, 10 mM Sodium Cacodylate buffer pH 7.00, 500 mM isopropanol, 100 nM polyamide (0.1% DMSO) and milliQ water (total volume

13  $\mu$ L) and incubated at room temperature for 1 hr. For UV irradiation LED light (model ZUVC30H manufactured by OMRON) with 300 mW at 365 nm was used. The reaction mixture was then treated with 1.25 unit of Uracil DNA Glycosylase (UDG) and incubated at 37 °C for 1h. The sample was dried in spin vacoum pump. About 6  $\mu$ L loading dye was added to the sample and heated at 95 °C for 10 min.

## Analysis of Photoreacted Sample using PAGE

Before loading into the gel the photoreacted sample was mixed with 6  $\mu$ L of loading dye (loading dye was prepared by using 300  $\mu$ l of 0.5 M EDTA, 200  $\mu$ L of Milli-Q water, 10 ml of formamide and 2.5 mg of New fuchsin) and heated at 95 °C for 10 minute. After cooling down gradually, 1.5  $\mu$ L of the sample were loaded in a 6% denaturing polyacrylamide gel (7M Urea). It was then analyzed with sequencing ladder and detected by sequencer (SQ5500E, HITACHI). The sequencing ladder was prepared by Thermosequenase Dye Primer Manual Cycle Sequencing Kit protocol using the template DNA used in this study.

## **Optimization of Concentration**

In order to find out optimum condition for the photoreaction several concentrations of polyamide and irradiation period was optimized. We checked 0-500 nM range of polyamide concentration in 10 nM of DNA with 5 sec irradiation period and results shown in Figure S2 suggest 100 nM as the optimum concentration. While checking for the optimum irradiation time we used 0-60 sec on 100 nM polyamide and 10 nM DNA. The result in Figure S2 suggests 5 sec as the optimum irradiation period.

#### **SPR Analysis**

SPR analysis was performed using BIOCORE X instrument according to our previous report and following manufacture protocol (28,29). Biotinylated hairpin DNA was immobilized on streptavidin coated sensor chip SA to obtain the desired immobilization level (approximately 1200 RU rise after the loading of DNA on the sensor chip). The measurements was carried out using HBS-EP (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% Surfactant P20), purchased from GE Healthcare, with 0.1% DMSO at 25 °C. Different concentrations of polyamides sample were prepared in the HBS-EP buffer with 0.1% DMSO and injected at a flow rate of 20  $\mu$ L/min. Association rate (k<sub>a</sub>), dissociation rate (k<sub>d</sub>), and dissociation constant (K<sub>D</sub>) were calculated through data processing and fitting with 1:1 binding with mass transfer model using BIAevaluation 4.1 program. The closeness of fit is described by the statistical value  $\chi^2$ . Specificity was calculated by dividing mismatch/reverse with the match site. All the biotinylated DNA were purchased from JBios and their sequences are as follows: 5'-Biotin-GCGCTTGAGTCGCGTTTTCGCGACTCAAGCGC

5'-Biotin-GCGCATGTATCGCGTTTTCGCGATACATGCGC

5'-Biotin-GCGCTATGCTCGCGTTTTCGCGAGCATAGCGC

5'-Biotin-GCGCTTCACTCGCGTTTTCGCGAGTGAAGCGC

5'-Biotin-GCGCATACGACGCGTTTTCGCGTCGTATGCGC



**Figure S1.** The electron injection sites in DNA1 by Polyamides 1, 2, 3 and 4 are shown. The binding sites are shown in different colors: red indicates a match site, blue indicates a 1 bp mismatch site the boxes at mismatch sites indicate the mismatch bp and arrows indicates cleavage intensity observed.



**Figure S2. (A)** Slab gel sequencing analysis of a 6% denaturing (7 M urea) polyacrylamide gel for the DNA2 (298 bp) after photoreaction using polyamides **1–4**. Lanes a–e, photoirradiation period of 0, 5, 10, 15, and 30 s, respectively, at 365 nm UV using 10 nm DNA and 100 nm polyamide. Top- and bottom-strand analyses are shown on the left and right, respectively.



Figure S2 (B) Mapping of the electron-injection site from the gel shown in Figure 4A for the DNA1 in both the top and bottom strands. (C) Electron-injection sites for polyamides 1, 2, 3, and 4 at sites 1-6, 7–11, 12–14, and 15–17 respectively. The binding sites are shown in different colors: red indicates a match site, blue indicates a 1 bp mismatch site, and green indicates a reverse binding site; the boxes at mismatch sites indicate the mismatched base pair. Arrows indicate the strand-break site, with intensity.



**Figure S3.** Optimization of polyamide concentration and irradiation period. a) Slab gel sequencing analysis of a 6% denaturing (7 M urea) polyacrylamide gel for the DNA2 after photoreaction using polyamides **2**. Lane 1-6, polyamide concentration from 0, 50, 100, 150, 200, 300 and 500 nM respectively, irradiation period 5 s (at 365 nm) and 10 nm DNA. b) Optimization of irradiation period using 100 nM polyamide, 10 nM DNA. lane 1-5, irradiation period 0, 10, 30, 45 and 60 s. The result suggested 100 nM polyamide and 5 s irradiation is optimum.



**Figure S4.** Photo reaction was performed at different conditions to check the effect of uracil DNA glycosylase (UDG) and isopropanol (<sup>i</sup>PrOH) in the photo reaction. The conditions are UDG(-)<sup>i</sup>PrOH(-), UDG(-)<sup>i</sup>PrOH(+), UDG(+)<sup>i</sup>PrOH(-) and UDG(+)<sup>i</sup>PrOH(+). Plus indicates presence and minus indicates absence of it. A 1.25 U of UDG and 500 mM of <sup>i</sup>PrOH were used. Lane 1, 2 and 3: irradiation for 0, 10 and 30 sec. It has been noticed that presence of UDG and <sup>i</sup>PrOH facilitates the strand cleavage. As seen from the photoreaction, without UDG no strand cleavage was observed in both conditions (presence and absence of <sup>i</sup>PrOH). But the strand cleavage observed in the presence of UDG without <sup>i</sup>PrOH has pointed out the possibility of

DMSO present in the reaction mixture (polyamide solution containing DMSO) may act as hydrogen donor.



#### Effect of DMSO (may act as H-donor)

Figure S5. HPLC analysis of the photoirradiated sample of DNA (ODN1&2) containing hot spot sequence CAA<sup>Br</sup>UT. We confirmed the effect of <sup>i</sup>PrOH or DMSO in the reaction mixture which serves as hydrogen atom donor. Reaction condition without H-Donor: DNA (20 µM), Sodium Cacodylate pH 7.00 (10mM), Milli Q water, UV 302 nm for 1 h. Reaction Condition with H-Donor: DNA (20 µM), Sodium Cacodylate pH 7.00 (10mM), PrOH (500 mM) or DMSO (2 M) Milli Q water, UV 302 nm for 1 h. The photo irradiated sample was then subjected for the HPLC analysis. Elution was performed with 50 mM Ammoniumformate (pH 6.6) containing 0-10% acetonitrile over a linear gradient for 30 min at a flow rate of 1.0 mL/min at 40 °C. The HPLC column used for this experiment was COSMOSIL 5C18-AR-II 4.6ID x 150mm (nacalai tesque). Generally, irradiation of hot spot containing DNA generates 2-deoxyribonolactone with concomitant release of A base in absence of hydrogen atom donor as shown in figure B but the presence of hydrogen atom source quench the Uracil-5-yl radical species to Uracil without any release of A.1 In this experiment, Oligo 2 generates uracil containing product in presence of PrOH without release of A, shown in Figure D. Interestingly, the result indicates possibility of DMSO as hydrogen donor. In the presence of DMSO many peaks appeared and one of which was Uracil containing product. Also there was no release of A as shown in Figure C. This clearly

indicates that DMSO can also act as hydrogen donor and this is possibly the reason behind strand cleavage observed in absence of <sup>i</sup>PrOH but presence of UDG shown in Figure **S4**.



**Figure S6.** Photo reaction was performed using polyamide without having pyrene as shown 2` and polyamide with pyrene as 2 in order to clarify whether 2` can inject electron or not. From the photo reaction it was found that 2` could not inject electron whereas 2 injected electron to <sup>Br</sup>U residues at the binding site (DNA2, top strand) under photo irradiation condition. This is consistent with our previous report.<sup>2</sup>





 $K_{\rm D} = 1.05 \times 10^{-7} \text{ (M)}$   $k_{\rm a} = 2.35 \times 10^{3} \text{ (M}^{-1}\text{s}^{-1}\text{)}$  $K_{\rm d} = 2.46 \times 10^{-4} \text{ (s}^{-1}\text{)}$ 



d)

Match 2





**Figure S7.** SPR analysis of pyrene polyamides. a) Polyamide 1 at match site shown in red. b) Polyamide 1 at 1 bp mismatch site shown in blue and the box indicates the mismatch base pair. c) Polyamide at reverse binding orientation shown in green d) Polyamide 2 at match site shown in red e) polyamide 2 at reverse binding orientation shown in green. X-axis indicates running time and Y-axis is the corresponding RU.

Reference:

- Hashiya, F., Saha, A., Kizaki, S., Li, Y., Sugiyama, H. Nucleic Acids Res., 2014, 42, 13469-13473
- 2. Morinaga,H., Takenaka,T., Hashiya,F., Kizaki,S., Hashiya,K., Bando,T., Sugiyama,H. Nucleic Acids Res., 2013, 41, 4724-4728.