# G-quadruplex-guided bifunctional platinum complex induce multiple pyroptosis pathways for antitumor therapy

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**Experimental**

**DNA sample preparation.** The oligonucleotides were purchased from Sangon Biotechnology (China) and purified by HPLC. The DNA sequences used in this study are shown in Table S1. The G-quadruplex (G4) structure was formed by heating at 95 °C for 5 min, followed by slow cooling in buffer to room temperature overnight. The DNA concentration was measured by a Nanodrop 200/200c (Thermo Science).

**Purity analysis by LC-MS**. Analytical HPLC was used for analysis with an injection volume of 10 μL. Phase A is $H_2O$ with 0.1% $CH_2O_2$, and phase B is methanol ($CH_3OH$) with 0.1% $CH_2O_2$. The absorbance wavelength was set to 254 nm. 0-10min: 5% B-100% B; 10-15 min: 100% B. The absorbance wavelength was set to 254 nm. sample was analyzed by HPLC (Thermo Fisher) with a C18-H HPLC column (4.6 × 250 mm, 5 μm, 120 Å) at a flow rate of 1.0 ml/min and column temperature of 25 °C. The retention times were liste as follows: **BIPP** is 10.487 min.

**Fluorescence resonance energy transfer (FRET) melting assay.** DNA was fluorescently labeled with FAM (6-carboxyfluorescein) at the 5'-end and TAMRA (6-carboxytetramethylrhodamine) at the 3'-end for FRET melting assays. The DNA sequences used are shown in Table S1. Fluorescent DNA samples (c-MYC G4 DNA, VEGF G4 DNA) were prepared in 25 mM potassium phosphate and 70 mM KCl buffer (pH 7.4) and 22AG was prepared in 25 mM sodium phosphate and 70 mM NaCl buffer (pH 7.4). MYT1L G4 DNA was prepared in 10 mM Tris-HCl buffer (pH 7.4, 40 mM KCl). **BiPP** and **PyPDS** respectively together with the G4s and dsDNA incubation 48 h. Fluorescent melting curves were obtained using a Roche Light Cycler 2.0 real-time PCR instrument with an excitation wavelength of 470 nm and a detection wavelength of 530 nm. The data were read in the range from 37 to 99 °C once every 1 °C rise, and a constant temperature was maintained for 30 s before each reading. We first drew the curve of $\Delta T_m$ and molar ratio (G4/ ds DNA) from the original data, and then calculated the $\Delta T_m$ of each G4/ ds DNA, the ratio of $\Delta T_m$ of the G4 and complex system with ds DNA (ds-12CT) to the $\Delta T_m$ of the G4 and complex system without ds DNA was calculated to obtain % Stabilization. For competition experiments, different proportions of dsDNA

were added to **BiPP** (1600 nM) with c-MYC, VEGF or MYT1L G4 DNA (400 nM) systems for competition. **Polyacrylamide gel electrophoresis (PAGE)**. MYT1L G4 DNA was prepared in 10 mM Tris-HCl buffer (pH 7.4, 40 mM KCl). 22AG was prepared in 25 mM sodium phosphate and 70 mM NaCl buffer (pH 7.4). G4 DNA and dsDNA samples incubated with different ratios of **BiPP** and monofunctional platinum for 48 h. Native PAGE experiments were carried out with a native gel containing 15% acrylamide in 1x TBE buffer (pH = 8.0). G4 DNA and dsDNA samples for denatured urea-PAGE assays were prepared in 10 mM Tris-HCl buffer (pH 7.4, 40 mM KCl) incubated with different ratios of **BiPP** and monofunctional platinum for 48 h, and then added the urea-PAGE loading buffer and heated at 95 °C for 5 minutes before cooling on ice. Denatured urea-PAGE experiments were carried out with a native gel containing 15% acrylamide and 0.5g/mL urea in 1x TBE buffer (pH = 8.0). DNA bands were dyed by ethidium bromide (EB), and photographed by FluorChem M imager (ProteinSimple).

**Mass spectrometry.** MYT1L, 22AG G4 DNA were prepared with 40 mM ammonium acetate buffer (pH 7.4). The concentration of DNA was 30 μM. **BiPP** were added. The mixture was incubated for 48 h at 37 °C and centrifuged with AMICON ULTRA 0.5ML 3K 96PK, and the filtrate was collected. A German LTQ Orbitrap Elite mass spectrometer was used for negative spray ionization mass spectrometry.

**LC-MS Analysis.** All DNA oligonucleotides used in Table S1. The oligonucleotides were annealed at a concentration of 400 nM, in 100 mM KCl, Tris.HCl buffer, pH 7.4, by heating at 94 ºC for 10 min followed by slow cooling to rt at a controlled rate of 0.1 ºC/min. Annealed oligonucleotides were incubated with different doses of **BiPP** in the same buffer at 37 ºC for 1 h. Incubated DNA was nuclease digested as by a literature protocol, 24 purified with Amicon Ultra 0.5 mL 3 kDa columns and analysed by Agilent Technologies 1290 Infinity II 6545 Q-TOF LC/MS. The system was equipped with an Eclipse Agilent column EC-C18 3.0 x 150mm. The column temperature was maintained at 45°C. Eluting buffers were buffer A (500 mM Ammonium Acetate (Fisher) pH 5), Buffer B (Acetonitrile) and Buffer C ($H_2O$). Buffer A was held at 1% throughout the whole run and the gradient for the remaining buffers was 0 min – 5% B, 20 min – 50% B, 40 min – 80% B, 45 min – 95% B.

**Fluorescence lifetime imaging microscopy (FLIM).** FLIM imaging was performed using a Zeiss LSM 880 NLO multiphoton microscope equipped with a bh TCSPC FLIM system (Becker & Hickl GmbH, Berlin, Germany). Samples were two-photon excited using a femtosecond Ti:Sapphire laser (Coherent Chamole), and emitted light was collected using a Zeiss BiG-2 GaAsP detector. The TCSPC module type is the SPC-150 module, and the image resolution is 512×512 pixels. The G4 probe **NBTE** (20 μM) was incubated with HeLa cells for 18 h at 37 °C. They were then further incubated with **BiPP** (5 μM) at 37 °C for 0 h, 6 h, 12 h or 36 h. Then, they were washed 3 times with ice-cold PBS buffer and detected with the FLIM system. The two-photon excitation wavelength of **NBTE** was 810 nm, and the emission was recorded from 545 nm to 590 nm. The acquisition time was sufficient, and the signal-to-noise ratio was good.

Lifetime data were fitted to a three-exponential function for each pixel using SPC-Image software. For lifespan distributions, approximately 100 cells were analyzed in each sample. Images are representative of repeated experiments (n=3). The percentage of G4 peaks was calculated by dividing the G4 peak area (3.0 to 5.0 ns) by the total area (0 to 5.0 ns).

**Platinum level in cells and genomic DNA.** MDA-MB-231 cells were incubated for 12 h in a 10 cm dish. To observe the accumulation of platinum complexes in cells, after 24 h of treatment with the indicated concentrations of **BiPP** and cisplatin, cells were harvested and washed three times with PBS. Nuclei were isolated using a nuclear protein extraction kit (C500009, Sangon Biotechnology, China) according to the manufacturer's instructions. Mitochondria were isolated using a mitochondrial protein extraction kit (20128ES50, YEASEN). The cells or nuclei were lysed with radioimmunoprecipitation (RIPA) buffer, and the protein concentration was determined by the bicinchoninic acid (BCA) method. For platinum levels in genomic DNA, genomic DNA was extracted by a GeneJET Genomic DNA Purification Kit (Thermo Fisher) after 24 h of **BiPP** and cisplatin treatment. The DNA concentration was determined by a Nanodrop 200/200c (Thermo Science). The mixture of total cell, nuclear, mitochondrial, cytoplasmic or genomic DNA was digested in 400 μL 60% $HNO_3$ solution at 60 °C for 1 h and diluted to 10 mL with Milli-Q water (containing 10 ppb platinum as an internal standard), and the platinum concentration was determined by XSERIES 2 ICP–MS.

**Antitumor capacity and the effects of different inhibitors on cell death induced by complexes.** The antiproliferation activities of **BiPP, CP** and **PyPDS** *in vitro* were detected by the methyl thiazol tetrazolium (MTT) assay. Cells were first seeded in 96-well microplates and incubated for 24 h at 37 °C in a cell incubator with 5% $CO_2$. We then incubated the cells with the test concentration of the complex. The incubation was continued for 44 h, and then MTT solution (20 μL, 5 mg/mL, dissolved in 1× PBS) was added to each well. After 4 h of incubation, the culture was removed, and 150 μL of DMSO solution was added to each well. The optical density of each well was measured with a microplate spectrophotometer at a wavelength of 595 nm. The percentage of cell viability was calculated using the following formula: (mean OD value of cells in the treatment group/mean OD value of cells in the control group) × 100%. Cells treated with solvent control (1% DMSO) were used as the control group. Data are presented as the mean ± standard deviation (n = 3). To evaluate the effect of different inhibitors on cell death induced by **BiPP, CP** and **PyPDS**, inhibitors (Nec-1: 100 μM; Z-VAD-FMK:10 μM; NSA:10 μM and 2-BP: 50 μM) were incubated for 1 h and then coincubated with **BiPP, CP** and **PyPDS** for 48 h. Each experiment was repeated at least three times to obtain the mean value.

**Scanning electron microscopy.** MDA-MB-231 cells were incubated with DMSO and **BiPP**, **PyPDS** for 24 h, and then the cells were fixed with 4% electron microscope fixable solution at 4 °C overnight and washed three times with PBS. The samples were dehydrated by 30, 50, 70, 80, 90 and 100% ethanol,

dried by the tert-butyl alcohol method, and imaged by scanning electron microscopy at 5.0 kV.

**Whole genome sequencing, transcriptome sequencing and data study.** MDA-MB-231 cells were incubated in 10 cm dishes for 12 h and then treated with **BiPP** (3.0 μM) and **PyPDS** (3.0 μM) for 24 h. DNA was collected for whole-genome sequencing. Total RNA was extracted with TRIzol reagent (Life Technologies, USA), and mRNA was purified with poly-T oligo-attached magnetic beads and used for RNA-Seq. Sequencing was performed by Biomarker Technologies. Whole-genome sequencing was performed according to standard procedures provided by Illumina. Raw reads were assessed for quality and filtered to obtain clean reads for downstream bioinformatics analysis. Sequencing reads were relocated to the human reference genome by BWA software. A deletion containing a fragment of $(G_nN_x)_4$ ($n \geq 2$, n denotes any base) was defined as a G4-mediated gene deletion and named G4Del compared to the control group.

For RNA-seq, libraries were sequenced on an Illumina NovaSeq platform to produce 150 bp paired-end reads. For quality control, Q20, Q30, GC content, and sequence repeat levels of clean data were calculated, and high-quality clean data were used for downstream bioinformatics analysis. The human genome (Homo_sapiens. GRCh38_release95. genome.fa) was used as the reference genome sequence and mapped by HISA2T. Gene expression levels were estimated from fragments per kilobase of transcript per million fragments mapped. DESeq2 was used to analyze differentially expressed genes (DEGs) between the two groups. DEGs were assigned as genes with a corrected p value ≤0.01 and fold change≥2 found by DESeq2.

**Immunofluorescence (IF) staining.** IF was performed as reported in the literature.[1] Briefly, MDA-MB-231 cells were seeded into 96-well plates, incubated for 24 h, treated with the indicated concentrations of **BiPP** and **PyPDS** and cisplatin for 24 h and washed three times with precooled PBS buffer. Cells were then fixed with 4% paraformaldehyde and permeabilized in 0.5% Triton X-100 (1×PBS). Cells were then incubated with anti-G-tetraploid (BG4) antibody for 2 h at room temperature. Subsequently, the cells were incubated with anti-Flag antibody and anti-γH2AX primary antibody, rabbit monoclonal to HMGB1, and rabbit monoclonal to calreticulin (CRT) antibodies overnight at 4 °C. Next, the cells were washed three times with PBST and incubated with secondary antibodies DyLight 549-conjugated anti-mouse and DyLight 647-conjugated anti-rabbit (Multi Sciences, China) for 1 h at 37 °C. The cells were stained with DAPI or Hoechst 33342. Fluorescence was detected using a 63× oil immersion objective confocal microscope (LSM 710, Carl Zeiss, Germany).

**Detection of cytosolic dsDNA in mitochondrial DNA-depleted HeLa cells ($\rho^0$).** MtDNA was removed by continuous incubation of HeLa cells for 3 weeks in medium containing EtBr (50 ng/mL), sodium pyruvate (110 mg/mL), uridine 50 (μg/mL), and glutamine (4 mM) as described in the previous relevant literature. Cells were then rested in the presence of uridine for 2 weeks to obtain mtDNA-depleted $\rho^0$ cells. Mitochondrial and nuclear gene expression was detected by real-time quantitative polymerase chain

reaction (RT–qPCR) to confirm the loss of mtDNA. HeLa $\rho^0$ cells were treated with **BiPP** and **PyPDS** for 24 h and then incubated with PicoGreen (1 μg/mL, 15 min) to detect the release of ncDNA from the cells. Images were observed by confocal microscopy. PicoGreen: $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 520 ± 20 nm.

**Western blot.** Western blotting was performed according to the manufacturer's protocol with slight modifications. Rabbit anti-GAPDH antibody (37 kDa) was used as a loading control. MDA-MB-231 cells were incubated with the indicated concentrations of **BiPP**, **CP**, **PyPDS** and cisplatin at 37 °C for 24 h and then collected and treated with RIPA lysis buffer containing 100 μg/mL PMSF for 30 min on ice. The samples were then centrifuged at 10000 × g for 15 min at 4 °C. The supernatant was added to loading buffer, heated at 100 °C for 10 min, and stored at -20 °C for further electrophoresis.

**Circular dichroism (CD) spectroscopy.** BIRC7 gene promoter G4 (Table S1) for CD spectra were recorded over a range of 200-400 nm (bandwidth = 1 nm, 1 nm data pitch, 1 s response, scan speed = 200 nm.min-1, averaged over 4 scans, zeroed at 400 nm). Samples were prepared in 300 μL (final volume) comprising G4 (5 μM final concentration) in 10 mM lithium cacodylate buffer (pH 7.2), 10 mM KCl and 90 mM LiCl without and with increasing amounts of **BiPP, CP** and **PyPDS** (1-5.0 eq). Final data were analyzed with Excel (Microsoft Corp.) and OriginPro®9.1 (OriginLab Corp.)

**qPCR stop assay**. According to the previous literature.[2, 3] For qPCR stop assays, templates (G4-strand and non G4-strand oligonucleotides) were prepared in the same way that CD. Polymerase reactions were carried out in triplicate in 96-well format using a Mx3005P qPCR machine (Agilent) equipped with FAM filters (lex = 492 nm; lem = 516 nm) in 10 μL (final volume). To a 1.35 μL solution of 0.5 μM template oligonucleotide (in 10 mM KCl) were added 3.15 μL or 2.65 μL of 380 mM KCl for experiments without molecule (control) or experiments performed in the presence of molecules respectively (around 100 mM KCl finale concentration). Next, 0.5 μL of the appropriated 6 μM primer were added (G4 Reverse primer for the G4-strand template and G4 Forward primer for the non G4-strand template), then 0.5 μL of 1.35 μM, 2.7 μM or 6.75 μM molecules (1, 2 or 5 mol. equiv., respectively, in 10 mM KCl) and 5 μL of iTaq™ Universal SYBR® Green Supermix (Bio-Rad). After a first denaturation step (95 °C, 5 min), a two-step qPCR comprising a hybridization step (85 °C, 10 s) and an elongation step (60 °C, 15 s) for 33 cycles was performed, and measurements were made after each cycle. Final data were analyzed with OriginPro®9.1 (OriginLab Corp.). The starting emission (first qPCR cycle) of SYBR Green (FI) was set to 2200 and the FI at the 33th cycle was used for calculation. Two biological triplicates (n= 6) were used. For statistical hypothesis tests Student's *t*-test was used.

**Real-time quantitative PCT (RT–qPCR).** RT–qPCR experiments were performed in the MDA-MB-231 cell line. The RNA was extracted with an EZ-press RNA Purification Kit, and the genome was removed and transcribed in one step with a Color All-in-one Reverse Transcription Kit (with DNase). qPCR was performed by adding the template ctDNA, primers and dd$H_2O$ to the color qPCR kit (premixed ROX1).

Real-time quantitative PCR (RT–qPCR) was performed in triplicate using a LightCycler 480 system (Roche Diagnostics, USA). The following primers were used: c-MYC (forward primer: 5'-GCTGCTTAGAC-GCTGGATT-3'; reverse primer: 5'-TCCTCCTCGTCGCAGTAGA-3'), VEGF (forward: 5'-TGGGG-GAGCGTGTCAGAAT-3'; reverse: 5'-CCGCTTTAATTGTGTGATTGGAC-3'), BIRC7 gene promoter G4 (forward: 5'- TGTCCACAGTGTGCAGGAGACT-3'; reverse: 5'-GGCACTTTCAGACTGGACCTCT-3') and GAPDH (forward primer: 5'-GGTGGTCTCCTCTGACTTCAACA-3', reverse primer: 5'-GTTGCTG-TAGCCAAATTCGTTGT-3'). Melting curves were used to detect and control the specificity of PCR products. The $2^{-\triangle\triangle ct}$ method was used to calculate relative gene expression levels, where the amounts of MYC mRNA and VEGF mRNA were normalized to an endogenous reference (GAPDH) and ct represents the threshold cycle.

**HMGB1 and CRT levels detected by flow cytometry.** MDA-MB-231 cells were seeded into 6 cm dishes and cultured for 24 h in complete DMEM. Cells were incubated with the corresponding concentrations of **BiPP** and **PyPDS** for 24 h. Cells were carefully harvested and then fixed, permeabilized, blocked, and then incubated with Alexa Fluor®488 rabbit monoclonal HMGB1 or Alexa Fluor®647 rabbit monoclonal calreticulin solution overnight at 4 °C. They were then washed with PBST, resuspended in 500 μL of PBS solution, and analyzed by flow cytometry. The median of Alexa Fluor®-488 fluorescence (FITC channel detection)/Alexa Fluor®-647 fluorescence (APC channel detection) was used to determine the intracellular HMGB-1/CRT protein content of the analyzed population. FlowJo software was used to analyze the data from three independent experiments.

**Determination of extracellular ATP concentration.** MDA-MB-231 cells were incubated in 6-well plates and grown in complete DMEM for 24 h at 37 °C in a cell incubator with 5% $CO_2$. The cells were incubated with **BiPP** and **PyPDS** at the corresponding concentrations for 24 h, and the extracellular ATP concentration was detected by luciferase luminescence assay using an ATP assay kit.

**Synthesis and Characterization. PyPDS** was synthesized with reference to a previous study.[4] The synthesis of bifunctional PyPDSplatin (**BiPP**), $K_2PtCl_4$ (0.1 mmol, 1.0 eq.) and **PyPDS** derivative (0.1 mmol, 1.0 eq.) were prepared in MeOH (6 mL) under nitrogen protection at 35 °C in the dark and stirred for 12 h. After the reaction, the supernatant was filtered, the samples were dried under vacuum and purified by HPLC to give the product (20.0 mg, yield 11%). The subsequent in vitro and in vivo activity assays were also conducted by dissolving **BiPP** in 5% (v/v) DMSO, followed by dilution with other appropriate buffer solutions before conducting the experiments. ESI-MS (m/z): $[M+3H]^{3+}$ calcd. for $C_{41}H_{49}N_9O_5PtCl_2$, 337.58; found: 337.58. $[M+2H]^{2+}$ calcd. for $C_{41}H_{49}N_9O_5PtCl_2$, 506.32; found: 506.38. $[M+H]^{+}$ calcd. for $C_{41}H_{49}N_9O_5PtCl_2$, 1012.77; found: 1012.74. LC-MS purity = 98.6% at 254 nm. Elemental Analysis: calcd for $C_{41}H_{59}N_{11}O_{16}PtCl_2$: C, 40.10; H, 4.84; N, 12.55; found: C, 40.52; H, 4.38; N, 13.01. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.14 (s, 2H), 8.33 (d, $J$ = 8.0 Hz, 2H), 8.15 (d, $J$ = 8.0 Hz,

2H), 8.02 (s, 2H), 7.99 (d, *J* = 4.0 Hz, 2H), 7.97 (d, *J* = 4.0 Hz, 2H), 7.82 (t, *J* = 8.0 Hz, 2H), 7.58 (t, *J* = 8.0 Hz, 2H), 4.71 (s, 2H), 4.63 (m, 4H), 3.83 (m, 4H), 3.70 (b, 4H), 3.29 (s, 4H), 2.50 (s, 8H), 2.01 (d, 8H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 166.88, 163.76, 161.94, 152.78, 151.53, 147.53, 131.29, 127.34, 125.32, 123.25, 119.89, 112.90, 95.64, 66.79, 64.66, 53.81, 53.00, 48.74, 45.31, 23.16.

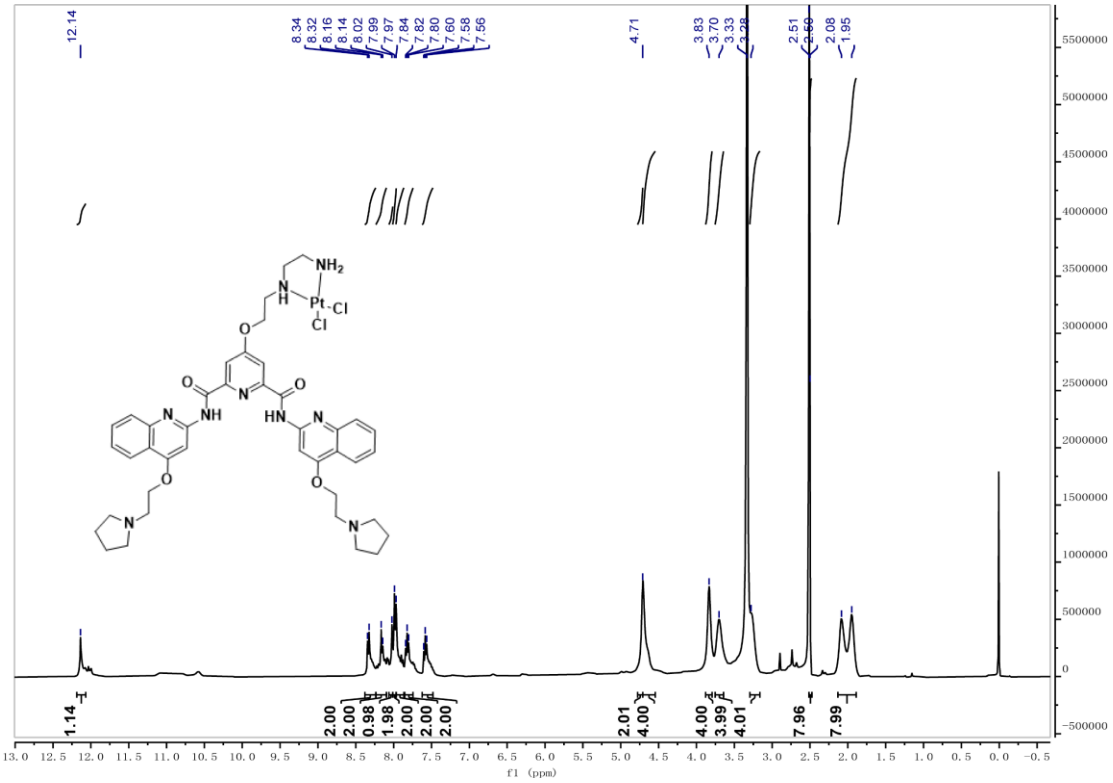



**Figure S1.** $^{1}$H NMR spectrum of **BiPP** in DMSO-$d_6$ measured immediately at 25 °C.

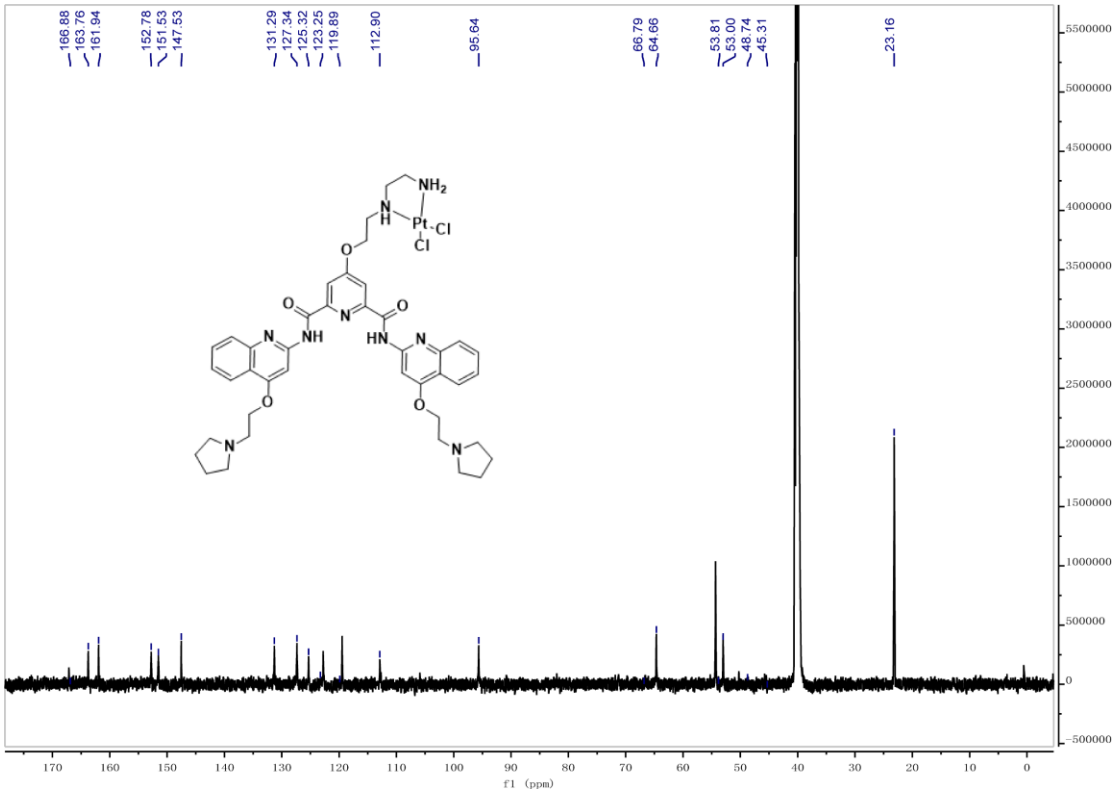


**Figure S2.** $^{13}C$ NMR spectrum of **BiPP** in DMSO-$d_6$ measured immediately at 25 °C.

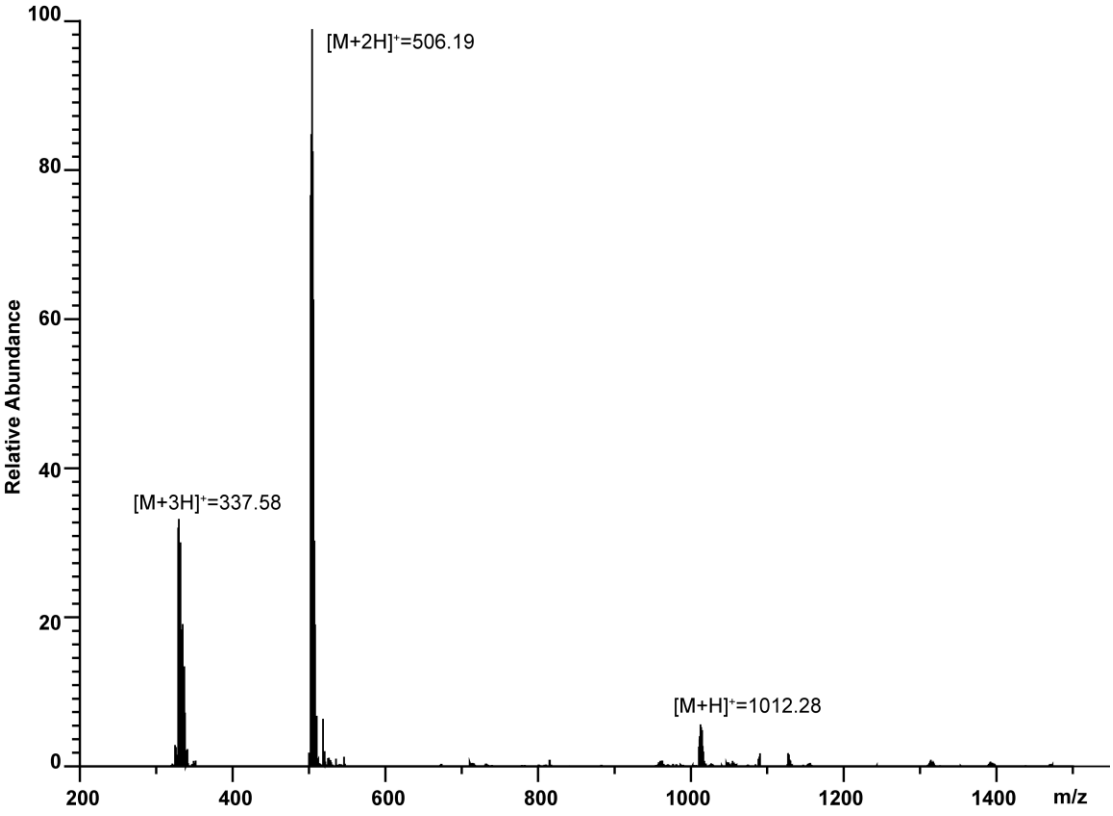
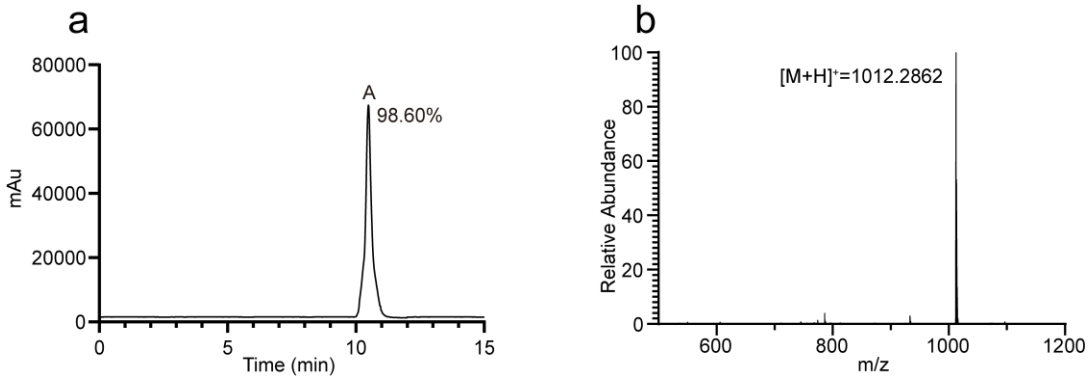



**Figure S3.** Mass spectrum of **BiPP**.




| **BiPP** peak | RetTime [min] | Width [min] | Area [mAu*min] | Height [mAu] | Area % |
|---|---|---|---|---|---|
| A | 10.487 | 1.388 | 42853.532 | 67538.271 | 98.60 |

**Figure S4.** Purity of **BiPP** analyzed by LC-MS. (a) LC-MS chromatograms and (b) mass spectra of **BiPP.**

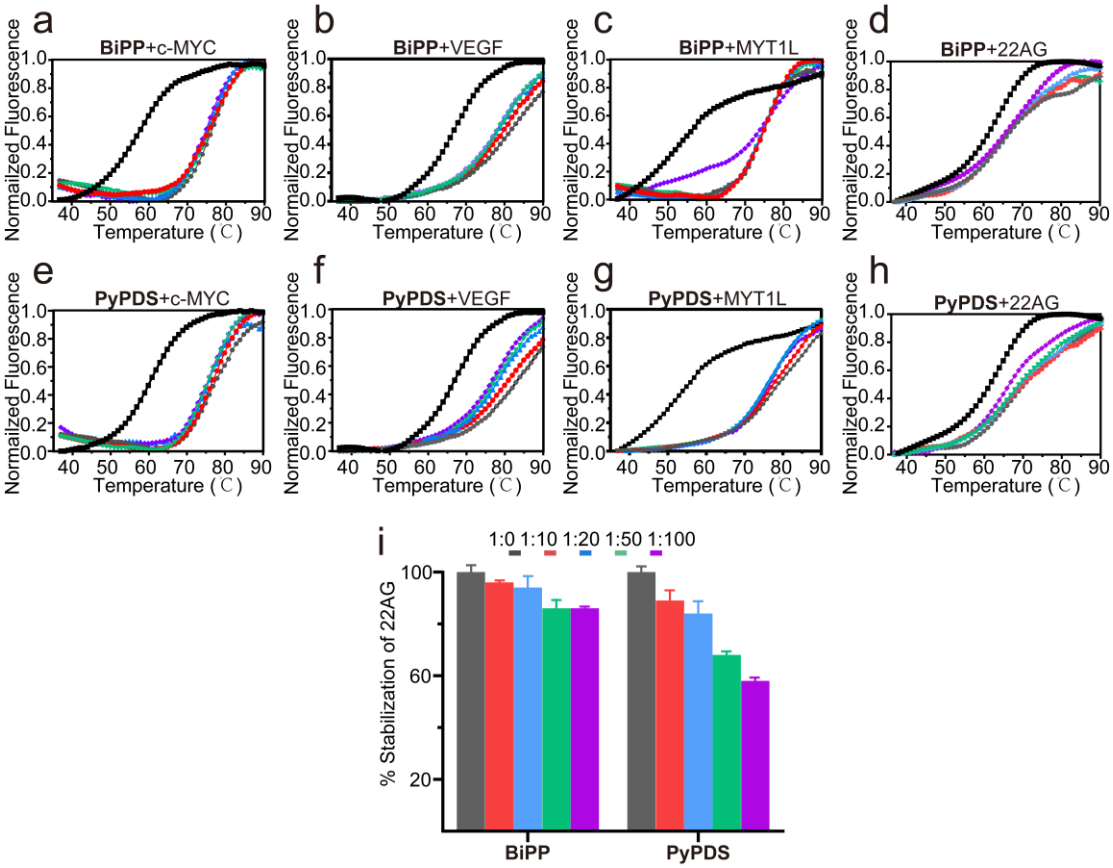



**Figure S5.** (a-h) FRET-melting curves of DNA samples were prepared in $K^+$ or $Na^+$ (pH 7.4). **BiPP** and **PyPDS** respectively together with the G4s and dsDNA incubation 48 h. Black represents the FRET-melting curve of free G4, grey, red, blue, green and purple represent the FRET-melting curves of G4 : ds DNA=1:0,1:10,1:20,1:50 and 1:100, respectively. (i) Thermal stabilization of 22AG with **BiPP** and **PyPDS** while competing with increasing ratios of ds-12CT for binding to ligands. After mixing the compound and DNA, the samples were tested immediately.

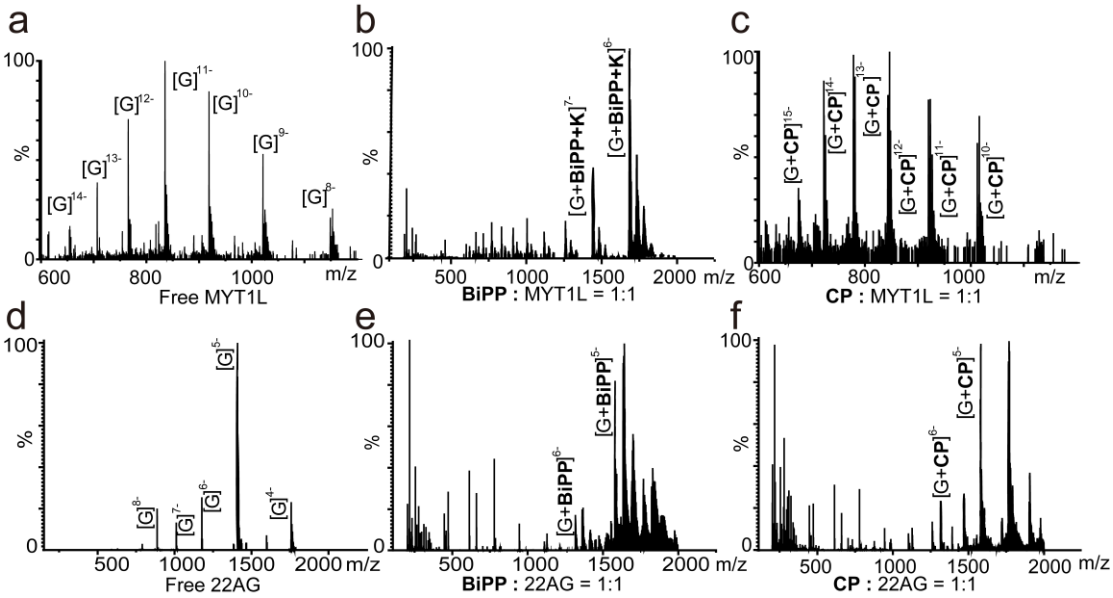



**Figure S6.** ESI-MS of MYT1L and 22AG interacting with **BiPP** and **CP**. The peaks are labeled with "G", meaning "MYT1L and 22AG", **Ligand:** G = 1: 1.

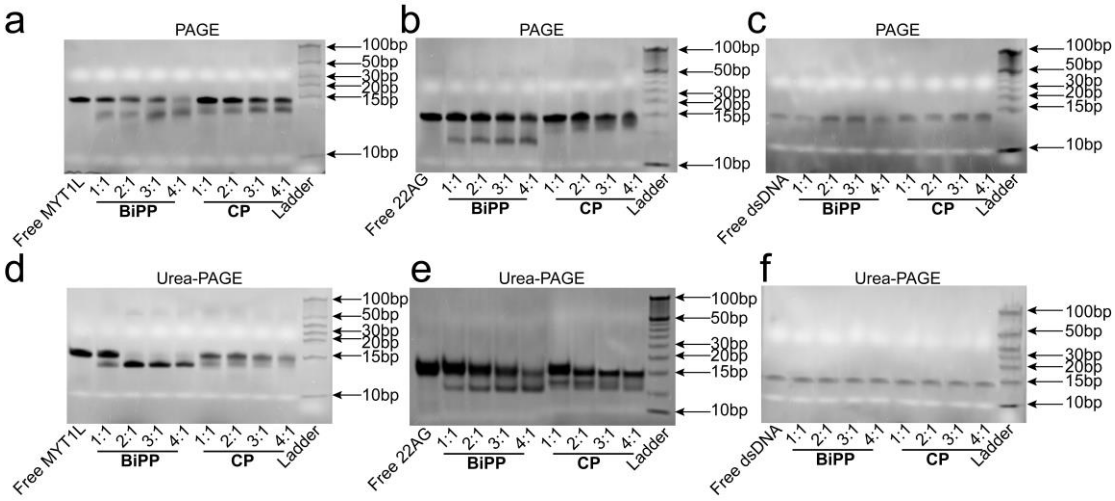



**Figure S7.** (a-c) The native PAGE and (d-f) denatured urea-PAGE of G4s and dsDNA incubated with different ratios of **BiPP** and **CP** for 48 h. The ratios of ligand/G4s are labeled.

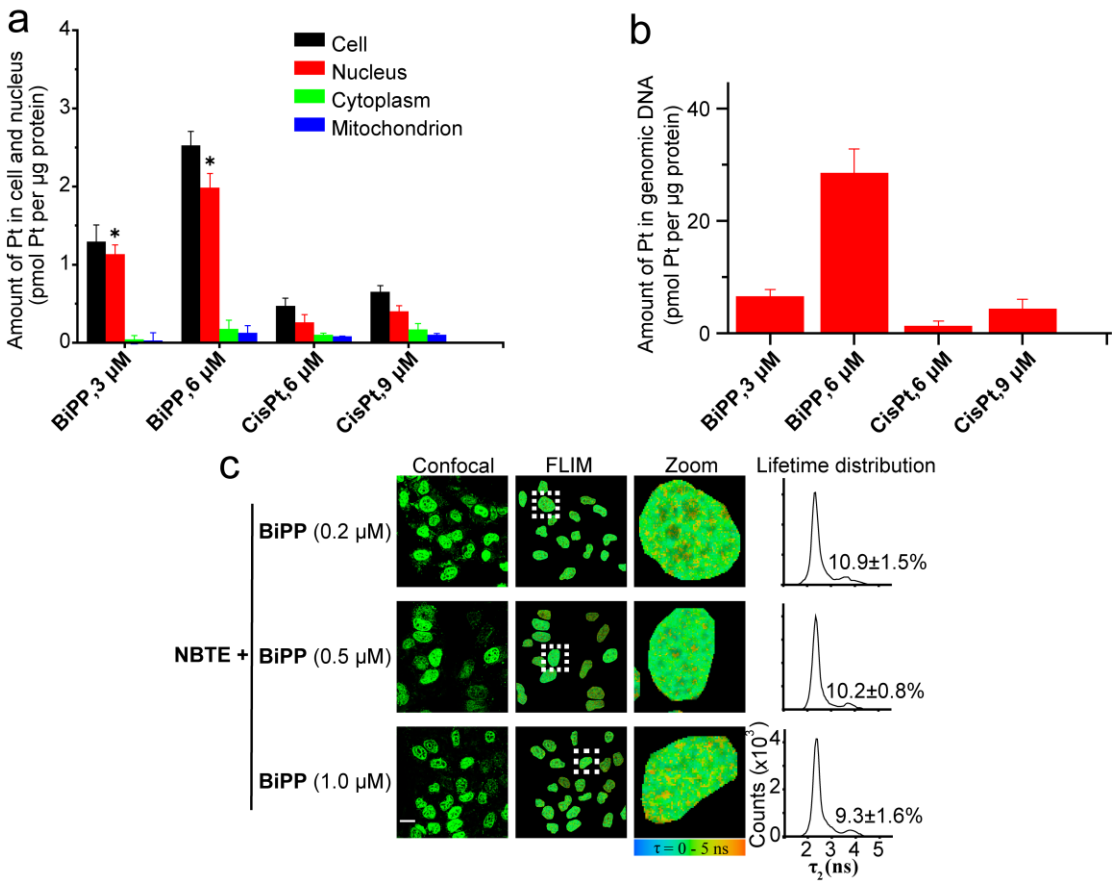



**Figure S8.** (a-b) The amount of Pt in the cell and nucleus and the amount of Pt in the genomic DNA of MDA-MB-231 cells after treatment with different concentrations of **BiPP** for 12 h. (c) FLIM experiments of live cells incubated with the DNA probe (**NBTE**, 20 μM, 24 h) while competing with **BiPP** for binding to G4s at 36 h. The results showed that **BiPP** could target G4s in live cells. Errors are s.d. (n = 3). Scale bar = 20 μm.

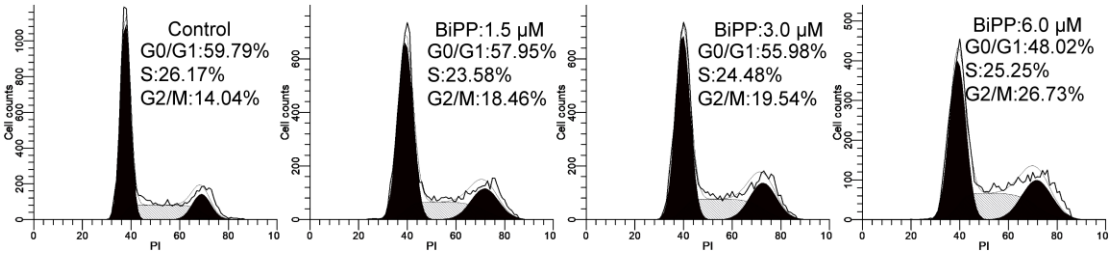



**Figure S9.** Quantitative cell-cycle distribution data for MDA-MB-231 cells after treatment with **BiPP** for 24 h analyzed by flowcytometry.

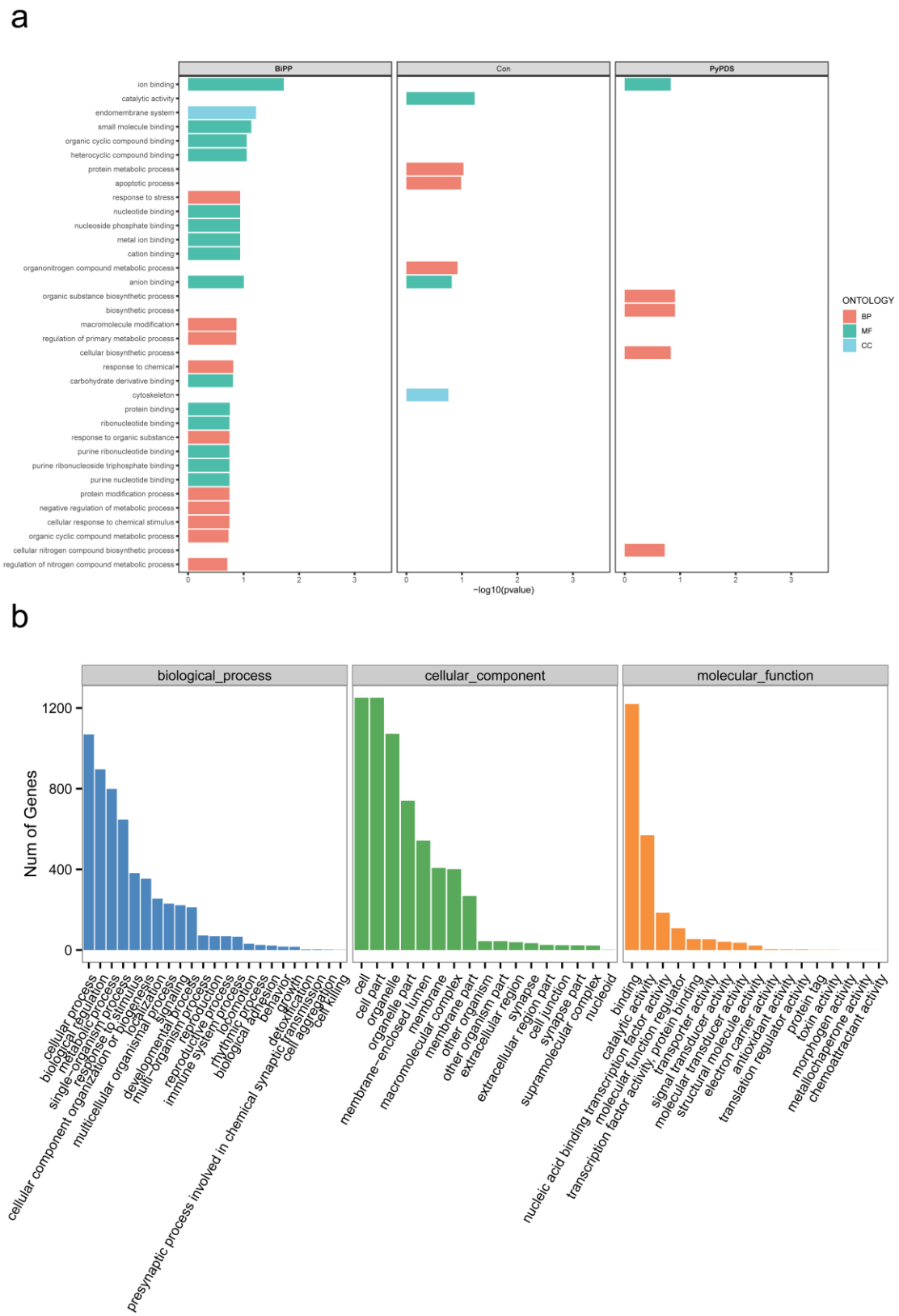



**Figure S10.** GO enrichment of target gene.

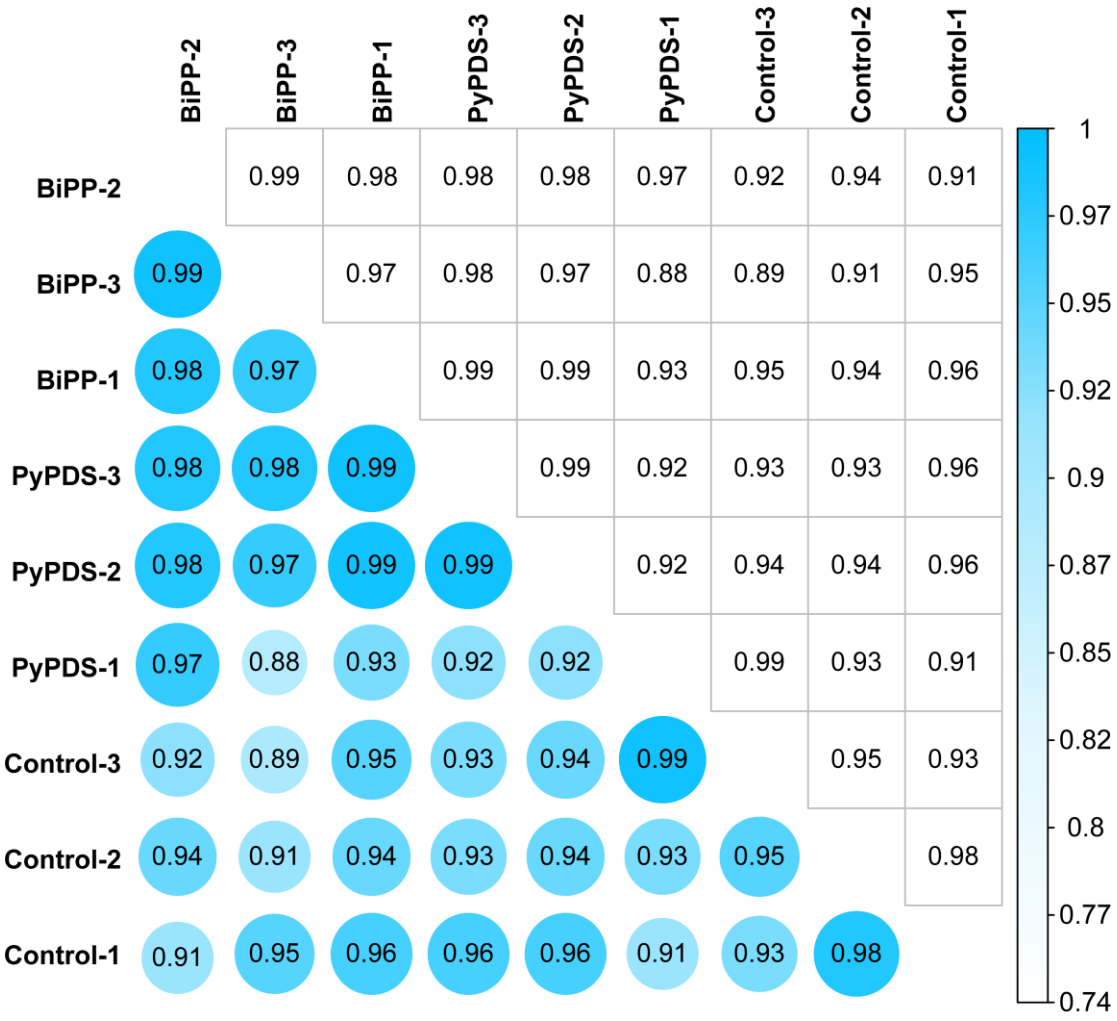



**Figure S11.** Heatmap diagram of Pearson correlation coefficients between the RNA-seq samples. Cell samples were treating with **BiPP**, and the reproducibility of the data was confirmed by correlation coefficients (above 0.80) between parallel samples.

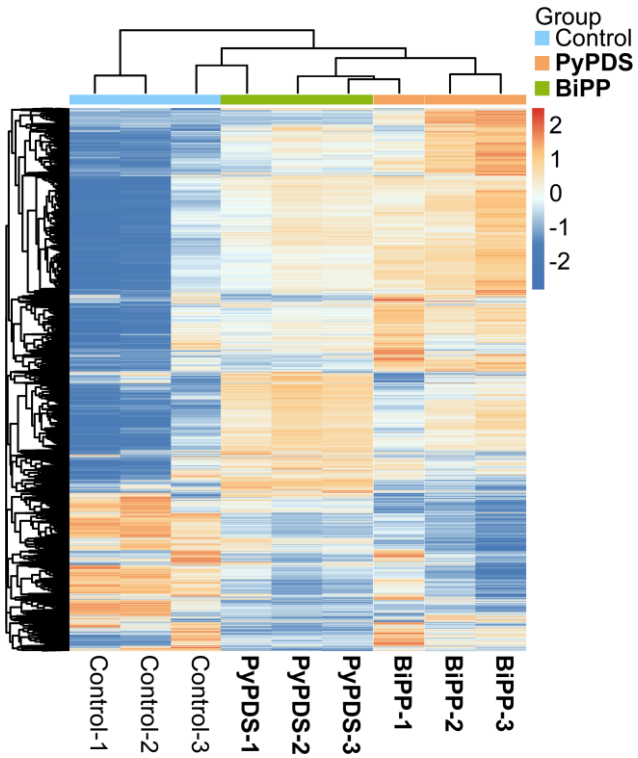



**Figure S12.** (a) Cluster analysis and Heatmap displayed the overview of the differentially expressed genes (DEGs) induced by **BiPP** and **PyPDS** for 24 h. RNA-seq clustering heatmaps showed that the expression patterns of transcription treatment groups of **BiPP** and **PyPDS** were similar, indicating that the cellular response mechanisms of **BiPP** and **PyPDS** group transcription were comparable.

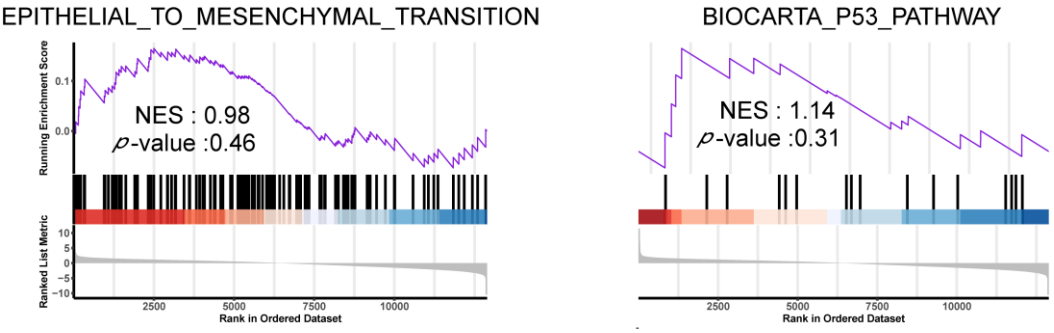



**Figure S13.** GSEA reveals positive enrichment of genes altered in cells subjected after **BiPP** treated.

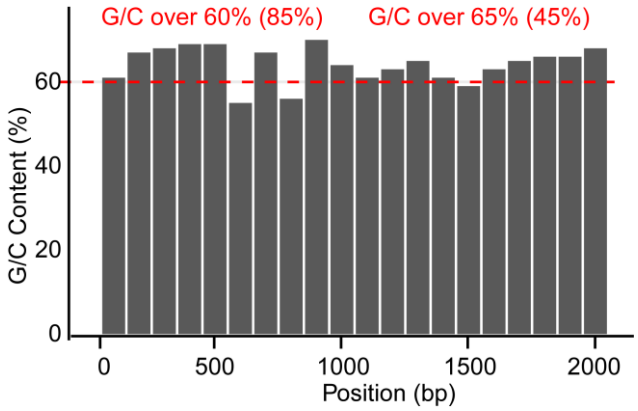



**Figure S14.** Bar graph of G/C content of G4 in the promoter region of the BIRC7 gene.

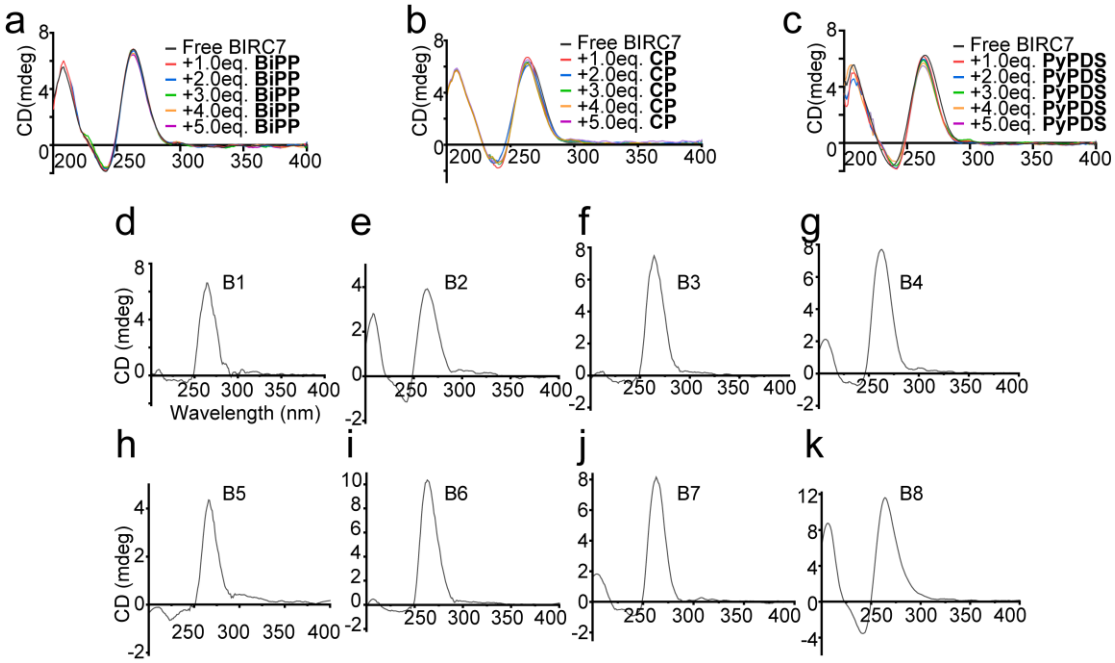



**Figure S15.** (a-c) CD spectra of G4Del sequence from whole genome sequencing interacted with **BiPP** and **CP** in 10 mM Tris-HCl buffer (pH 7.4, 100 mM $K^+$). The sequences are showed in Table S1. (d-k) BIRC7 gene promoter G4 showed parallel G4 conformation with a positive peak at 260 nm and a small negative peak at 240 nm. The ratio of **BiPP** to DNA.

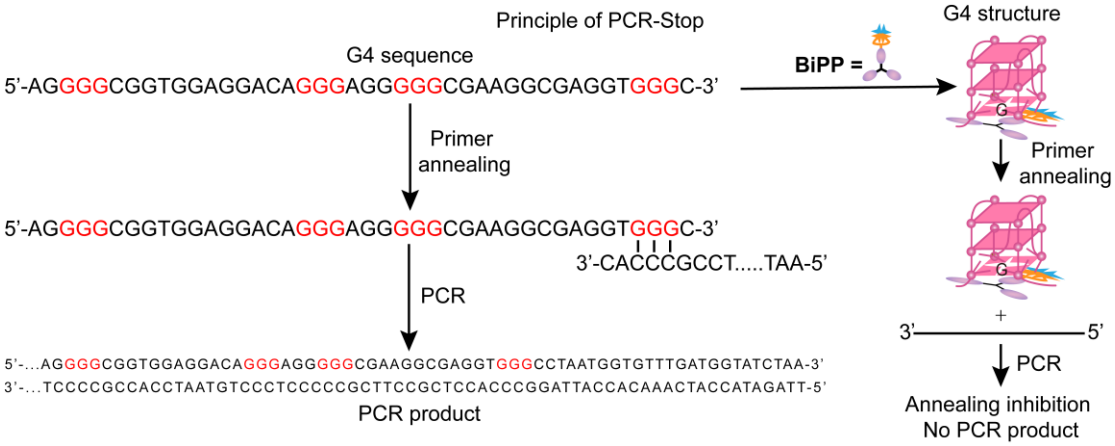



**Figure S16.** Principle of PCR-Stop analysis.

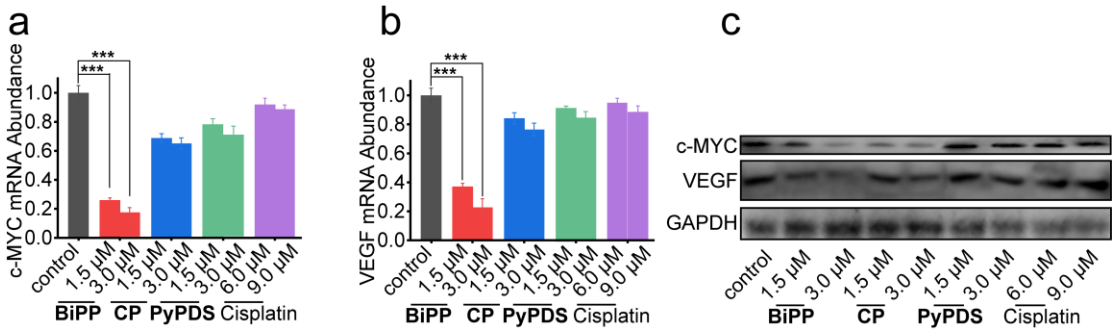



**Figure S17.** (a,b) The mRNA transcription levels of c-MYC and VEGF were inhibited after **BiPP, CP** and **PyPDS** treatment, as shown by RT–qPCR in the MDA-MB-231 cell lines. Errors are s.d. (n≥3). ***p<0.001.

(c) Western blot analysis showing the impact of **BiPP, CP** and **PyPDS** on the expression of the indicated proteins. Cells were treated with compounds for 24 h.

**Table S1**. List of DNA sequences used in this research.

| Name | Sequence (5' -> 3') |
|---|---|
| c-MYC | TGAGGGTGGGTAGGGTGGGTAA |
| VEGF | CGGGGCGGGCCTTGGGCGGGGT |
| MYT1L | AGGGAGAGGAGAGCTCTGGGTTGGGTGGG |
| ds-12CT | CTTTTGCAAAAG |
| ds-26 | CAATCGGATCGAATTCGATCCGATTG |
| BIRC7-G4 | TGGGTTTGCTGGGGTTGAGGGCT |
| B1 | AGGGAGGTGGAGGGAGATGGGGGGAA |
| B2 | AGGGAGATGGGGGAAAGGGGGA |
| B3 | TGGGGGAAGGGGGAAGCGGGA |
| B4 | AGGGGGAGCGGGAGGTGAGGGGCA |
| B5 | CGGGAGGTGAGGGCAGCTCGGGC |
| B6 | TGGGTTTGCTGGGGTTGAAGGGCT |
| B7 | TGGGGTTGAGGGCTCAGTCGGGGGT |
| B8 | AGGGCTCAGTCGGGGGTTGGGGGGT |
| G4-strand (qPCR stop) | TAGCCATTCAGCCGTAACAGGGGCGGTGGAGGACAGGGAGGGGGCGAAGGCGAGGTGGG CCTAATGGTGTTTGATGGTATCTAA |
| Non G4-strand (qPCR stop) | TTAGATACCATCAAACACCATTAGGTTCTACTG-TACTGCCCTGCCCTGCCCTGCCCTGTCTGTCTCTCTTCCACTGCCTGTTACGGCTGAATGGCTA |
| G4-Reverse (qPCR stop) | TTAGATACCATCAAACACCATTAGG |
| G4-Forward (qPCR stop) | TAGCCATTCAGCCGTAACAG |

**Table S2**. FRET-melting values of different systems of G4 (0.4 μM) with **BiPP** and **PyPDS** (1.6 μM) interacting with different equivalents of ds DNA in buffer solution (pH 7.4) containing $K^+$ or $Na^+$.

| G4 : ds DNA | **BiPP** | **PyPDS** |
|---|---|---|
| c-MYC : ds DNA = 1 : 0 | 21.3 ± 0.3 | 18.2 ± 0.5 |
| c-MYC : ds DNA = 1 : 10 | 21.0 ± 0.2 | 17.5 ± 0.6 |
| c-MYC : ds DNA = 1 : 20 | 20.9 ± 0.2 | 17.1 ± 0.7 |
| c-MYC : ds DNA = 1 : 50 | 20.7 ± 0.1 | 16.7 ± 0.1 |

| | | |
|---|---|---|
| c-MYC : ds DNA = 1 : 100 | 20.4 ± 0.1 | 16.4 ± 0.3 |
| VEGF : ds DNA = 1 : 0 | 14.2 ± 0.1 | 13.5 ± 0.5 |
| VEGF : ds DNA = 1 : 10 | 14.0 ± 0.2 | 13.0 ± 0.3 |
| VEGF : ds DNA = 1 : 20 | 13.9 ± 0.4 | 12.7 ± 0.3 |
| VEGF : ds DNA = 1 : 50 | 13.9 ± 0.1 | 11.6 ± 0.1 |
| VEGF : ds DNA = 1 : 100 | 13.8 ± 0.2 | 11.4 ± 0.2 |
| MYT1L : ds DNA = 1 : 0 | 20.5 ± 0.3 | 21.0 ± 0.4 |
| MYT1L : ds DNA = 1 : 10 | 20.3 ± 0.3 | 20.6 ± 0.2 |
| MYT1L : ds DNA = 1 : 20 | 20.1 ± 0.2 | 20.1 ± 0.5 |
| MYT1L : ds DNA = 1 : 50 | 20.0 ± 0.1 | 19.7 ± 0.1 |
| MYT1L : ds DNA = 1 : 100 | 19.5 ± 0.2 | 19.2 ± 0.3 |
| 22AG : ds DNA = 1 : 0 | 5.2 ± 0.8 | 8.9 ± 0.3 |
| 22AG : ds DNA = 1 : 10 | 5.0 ± 0.3 | 8.0 ± 0.1 |
| 22AG : ds DNA = 1 : 20 | 4.9 ± 0.5 | 7.5 ± 0.1 |
| 22AG : ds DNA = 1 : 50 | 4.5 ± 0.2 | 6.1 ± 0.4 |
| 22AG : ds DNA = 1 : 100 | 4.5 ± 0.2 | 5.2 ± 0.2 |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