# Supplementary Information

# for

## Development of Polymeric Aptamer Probes for In Vivo Continuous Precision Cancer Targeting

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### Reagents and materials.

All DNA oligonucleotides were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). DiO dye was purchased from Suzhou Yuheng Biotechnology Co. (Suzhoi, China). EDTA was purchased from Aladdin Industrial Co. (Shanghai, China). Exo I with 10× Exo I buffer were purchased from Takara Biotechnology Co. (Dalian, China).

### Cell lines and cell culture

All cells used in this experiment, including CCRF-CEM, MCF-7, A549, MDA-MB-231 cells were purchased from ATCC. CCRF-CEM and A549 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution at 37℃ in a 5% $CO_2$ atmosphere. MCF-7 and MDA-MB-231 were cultured in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution at 37℃ in a 5% $CO_2$ atmosphere.

### Washing buffer and binding buffer

Washing buffer was prepared with DPBS supplemented with 4.5 g/L of glucose and 5 mM of $MgCl_2$. Binding buffer was prepared with DPBS supplemented with 4.5

g/L of glucose, 5 mM of $MgCl_2$, 0.1 mg/mL of yeast tRNA, and 1 mg/mL of BSA.

**Methods.**

**Synthesis and characterization of PAPs**

AS1411 modified with azide groups ($N_3$-AS1411-$N_3$) and AS1411 modified with DBCO groups (DBCO-AS1411-DBCO) were added into DPBS at a molar ratio of 1:1 at a concentration of 5 μM, mixed well, and then reacted for 1 h at room temperature on a shaker, and then put into a refrigerator at -20 ℃ to continue the reaction for 12 h. Finally, it was taken out from the refrigerator and thawed, and then characterized by 8% PAGE.

**Stability analysis of aptamers in exonuclease solution and serum**.

2 μM Cy3-labeled monovalent aptamers and PAPs were incubated with 0.25 U/μl Exo I or RPMI 1640 with 10% fetal bovine serum (FBS) at 37 °C. At designated time points (0, 1, 2, 4, 8, 12, 24 h), samples were heated at 95 °C for 5 min to denature the enzyme and subsequently stored at -20 °C until all samples were collected. Samples were then thawed on ice for electrophoresis assays.

**Characterization of the G4 structure**

The non-fluorescently labeled single-chain AS1411 and PAPs were diluted to 2 μM with DPBS, and then 200 μL was taken on a circular dichroism spectrometer (MOS-500, Biologic, French) , and the measurement was repeated three times for each sample. Finally, the data were exported and statistically analyzed using origin 8.0.

**Flow cytometry assays**

(1) For adherent cells (MCF-7, A549, MDA-MB-231 cells), the adherent cells were firstly digested down and collected. Secondly, adding binding buffer and gently blown to prepare a homogeneous cell suspension. For each sample, 200 μL of cell suspension was added and the samples (Cy3-AS1411, Cy3-PAPs, and Cy3-Lib) were added to the tube (at a final concentration of 250 nM). The samples were mixed well and then put into the ice box to incubate for 45 min. After the incubation, all tubes were centrifuged and then washed twice with 200 μL of washing buffer to remove the unbound aptamer. Finally, the cells were resuspended into 200 μL of washing buffer for detection.

(2) For suspension cells, aspirate the cells directly from the dish into a centrifuge tube and centrifuge to remove the supernatant. Same as above for the rest.

**Confocal microscopy imaging**

The fluorescent dye was first dissolved in DMSO and configured into a 1 mM solution.**Incubate first and Stain later.** The CCRF-CEM cells were centrifuged at 800 rpm for 3 min, collected then washed twice with DPBS, aliquoted and resuspended into 200 μL of binding buffer and AS1411-Cy3 and PAPs-Cy3 (final concentration of 1 μM) were added, then put into an ice box and incubated for 45 min. After incubation, the cells were centrifuged, supernatant was removed, and then washed once with Washing buffer. The cells were then resuspended in 200 μL of DPBS and 1 μL of DiO dye (cell membrane dye, 488 nm excitation) was added, incubated at 37 ℃ for 10 min, centrifuged, supernatant was removed, and washed twice with washing buffer. Cell imaging was performed on an Airyscan confocal laser scanning microscope. **Stain first and incubate later.** Staining and incubation methods as above, order reversed.

**In vivo imaging**

All animal procedures were performed in accordance with protocol No. SYXK (Xiang) 2018-0006 approved by the Laboratory Animal Center of Hunan and experiments were approved by the Animal Ethics Committee of College of Biology (Hunan University).

Healthy 4- to 6-week-old female BALB/c nude mice were purchased from Hunan SJA Laboratory Animal Co.,Ltd. and $1\times10^7$ CCRF-CEM cells were injected subcutaneously into the right axilla of each BALB/c nude mouse. The tumors grow until the tumor volume reached about 300 $mm^3$, then BALB/c nude mice were randomly divided into groups and injected with 5 nmol of Cy5-labeled single-chain AS1411 or PAPs via the tail vein. At the indicated time points (0 h, 1 h, 2 h, 4 h, 6 h, 8 h), the mice were vaporized by respiration using an isoflurane vaporizer, and fluorescence images of the mice were captured using the IVIS Lumina II In Vivo Imaging System.

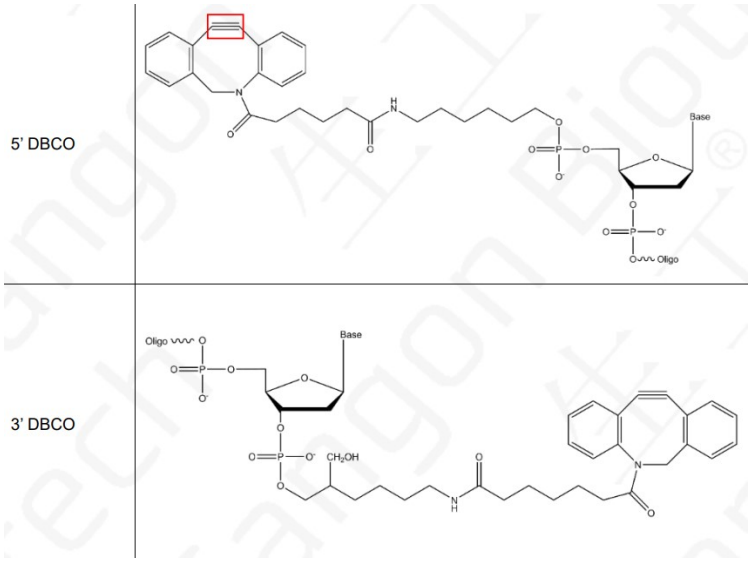
In both the experimental and control groups, one mouse was used per group. After tumor transplantation, the tumors were allowed to grow for 21–28 days until they

reached a volume of approximately 300 $mm^3$, at which point the experiment commenced. Imaging was conducted over an 8-hour period. No adverse effects were observed during the course of the experiment.

**Table S1:** Oligonucleotide sequences used in this study.

| Name | Strand components (5'-3') |
|---|---|
| Cy3-$N_3$-AS1411-$N_3$ | N3-GGTGGTGGTGGT (Cy3) TGTGGTGGTGGTGG-N3 |
| DBCO-AS1411-DBCO | DBCO-GGTGGTGGTGGTTGTGGTGGTGGTGG-DBCO |
| Lib | ATCTAACTGATTATTATTATTATTATTATTCGGTTAGA |
| Cy5-$N_3$-AS1411-$N_3$ | N3-GGTGGTGGTGGT (Cy5) TGTGGTGGTGGTGG-N3 |

**Figure S1**. Structure of $N_3$ and DBCO moiety.



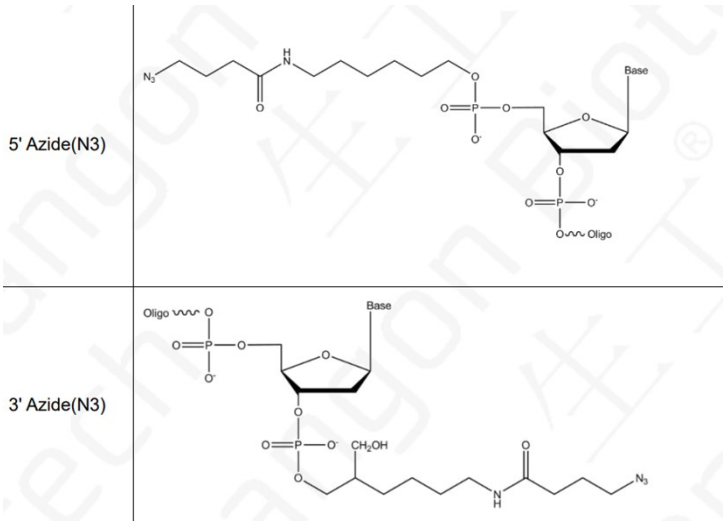



**Figure S2**. Determination of the average unit of polymerization.

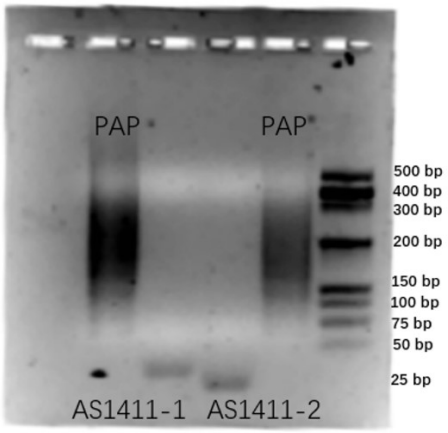
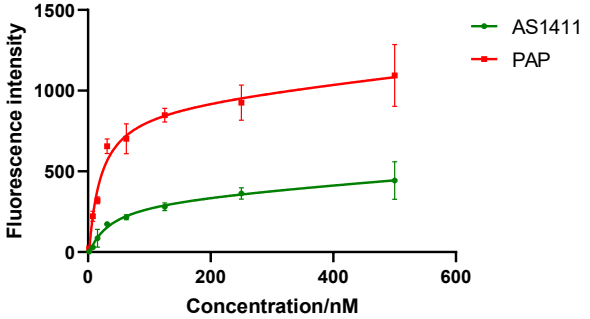
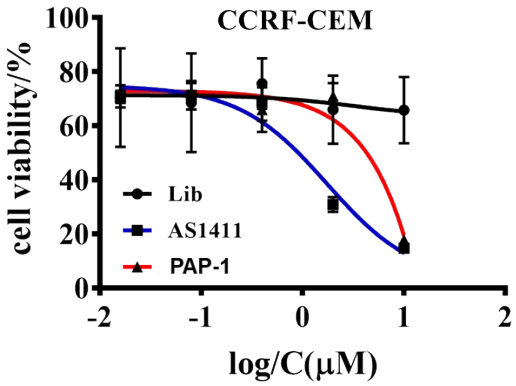



**Figure S3**. Determination of Kd values.




| | | A | B |
|---|---|---|---|
| | | AS1411 | PAP |
| 1 | **One site -- Total** | | |
| 2 | **Best-fit values** | | |
| 3 | Bmax | 357.0 | 1009 |
| 4 | Kd | 34.67 | 17.64 |

**Figure S4**. Determination of IC50 values




$IC_{50}$= 1.05 μM(AS1 411)

$IC_{50}$= 7.48 μM (PAP)