Supporting Information

Control of CRISPR-Cas9 with Small Molecule-Activated Allosteric Aptamer Regulating sgRNAs

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Experimental

In vitro target DNA cleavage assay.

The sgRNAs used in this assay were transcribed using a T7 High Yield RNA Synthesis Kit (NEB, America) according to the manufacturer's protocols. The products were purified with the GeneJET RNA Purification Kit (Thermo Fisher, America). The target DNA was amplified by polymerase chain reaction and purified using PCR Purification Kit (Sangon, China).

For the cleavage assay, 100 ng of the target DNA was incubated with 250 ng Cas9 protein in the presence of 200 ng of the sgRNA in the 1× Cas9 buffer (NEB, America). The *in vitro* reactions were incubated at 37°C for 2 h and analyzed by agarose gel electrophoresis.

Target DNA cleavage assay in cells.

HEK-293T cells were seeded at a density of 2×10^5 cells per well (24-well plate) in 1 mL growth medium (DMEM, 10% FBS), and allowed to attach overnight. Then the pGL3-Enhancer vector (Promega, America) and Cas9 plasmid (lentiCRISPR v2[1] containing sgRNA and sgB18-A30-S7 with luciferase guide sequence) were co-transfected using lipofectamine 3000 (Thermo Fisher, America) according to the manufacturer's protocols. After 6 h, the DMEM medium were refreshed and 1 mM theophylline was added to the medium. After transfection for 48 h, the cells were lysed using 100 μL lysis buffer and the fluorescence was tested using Luciferase Assay System (Promega, America). Twenty μL of the cell lysis were mixed with 50 μL substrate to test the fluorescence intensity.

Molecule Dynamics Simulation (MDS)

The structure of sgB18-A30-S7 was constructed using SimRNA.[2] Molecular docking between sgB18-A30-S7 and Cas9 as well as theophylline was modeled using Hex software.[3] MD simulations of the docking complex system were conducted using Gromacs 5.1[4]; the structure was minimized using bond constraints and run over 5000 steps of steepest descent and conjugate gradient minimization. The system was then gradually heated from 0 to 300 K in a canonical constant volume ensemble using Langevin dynamics with the collision frequency gamma_ln = 2.0 and position restraints on the solute molecules. Before production runs, 500 ps of NPT MDS without restraints was run at 300 K using Langevin dynamics with the collision frequency gamma_ln = 2.0. Finally, 10 ns MDS for the system was conducted at 300 K and at 1.0 atm. Electrostatics were handled using the particle mesh Ewald (PME) algorithm, and a 10.0 Å direct space was used as non-bonded cutoff. All bonds involving hydrogen atoms were constrained using the SHAKE algorithm with a time step of 2.0 fs. The coordinates of the trajectories were saved every 10 ps during the entire molecule dynamics run. MMPBSA[5] was used to calculate the Gibbs free energy change (ΔG) information (300 K, 1 atm).

Table S1. The sequences used in this work.

Name	Sequence（5’-3’ guide RNA not included）
EGFP Forward Primer	ATG GTG AGC AAG GGC GAG GA
EGFP Reverse Primer	TTA CTT GTA CAG CTC GTC CA
EGFP guide RNA	GGA GCG CAC CAU CUU CUU CA[6]

Table S2. The sequences used in this work.

Name	Sequence（5’-3’ guide RNA not included）
sgRNA	GUU UUA GAG CUA GAA AUA GCA AGU UAA AAU AAG GCU AGU CCG UUA UCA ACU UGA AAA AGU GGC ACC GAG UCG GUG C
sgB10-S5	GCU CUA AAA C**GA ACG GUU CCC GAC CAU A**GU UUU
sgB10-S7	GCU CUA AAA C**GA ACG GUU CCC GAC CAU A**GU UUU AG
sgB10-S8	GCU CUA AAA C**GA ACG GUU CCC GAC CAU A**GU UUU AGA
sgB12-S5	UAG CUC UAA AAC **GAA CGG UUC CCG ACC AUA** GUU UU
sgB14-S5	UCU AGC UCU AAA AC**G AAC GGU UCC CGA CCA UA**G UUU U
sgB16-S5	UUU CUA GCU CUA AAA C**GA ACG GUU CCC GAC CAU A**GU UUU
sgB18-S8	UAU UUC UAG CUC UAA AAC **GAA CGG UUC CCG ACC AUA** GUU UUA GA
sgB20-S8	GCU AUU UCU AGC UCU AAA AC**G AAC GGU UCC CGA CCA UA**G UUU UAG A
sgB22-S8	UUG CUA UUU CUA GCU CUA AAA C**GA ACG GUU CCC GAC CAU A**GU UUU AGA

Bold and underlined letters: the sequence of theophylline aptamer.

Table S3. The sequences used in this work.

Name	Sequence（5’-3’ guide RNA not included）
sgRNA	GUU UUA GAG CUA GAA AUA GCA AGU UAA AAU AAG GCU AGU CCG UUA UCA ACU UGA AAA AGU GGC ACC GAG UCG GUG C
sgB18-A30-S8	UAU UUC UAG CUC UAA AAC **AUA CCA GCA UCG UCU UGA UGC CCU UGG CAG** GUU UUA GA
sgB20-A30-S8	GCU AUU UCU AGC UCU AAA AC**A UAC CAG CAU CGU CUU GAU GCC CUU GGC AG**G UUU UAG A
sgB18-A30-S7	UAU UUC UAG CUC UAA AAC **AUA CCA GCA UCG UCU UGA UGC CCU UGG CAG** GUU UUA G
sgB18-A30-S6	UAU UUC UAG CUC UAA AAC **AUA CCA GCA UCG UCU UGA UGC CCU UGG CAG** GUU UUA
sgB18-A32-S7	UAU UUC UAG CUC UAA AAC **GAU ACC AGC AUC GUC UUG AUG CCC UUG GCA GC**G UUU UAG
sgB18-A32-S6	UAU UUC UAG CUC UAA AAC **GAU ACC AGC AUC GUC UUG AUG CCC UUG GCA GC**G UUU UA

Bold and underlined letters: the sequence of theophylline aptamer.

Table S4. The sequences used in this work.

Name	Sequence (5'-3' guide RNA not included)
Luciferase Forward Primer	CGC GAA TTC AAT GGA AGA CGC CAA AAA CAT AAA GA
Luciferase Reverse Primer	GAC TGG TAC CTT ACA CGG CGA TCT TTC CGC CCT TC
Luciferase guide RNA in vitro	AUA AAU AAC GCG CCC AAC AC[7]
Luciferase guide RNA in plasmid	GGA UUC UAA AAC GGA UUA CC[7]
TurboRFP Forward Primer	AGC GAG CTG ATC AAG GAG AA
TurboRFP Reverse Primer	GGT GAT TCT CTC CCA TGT GA
TurboRFP guide RNA	CGG AUG UGC ACU UGA AGU GG[8]

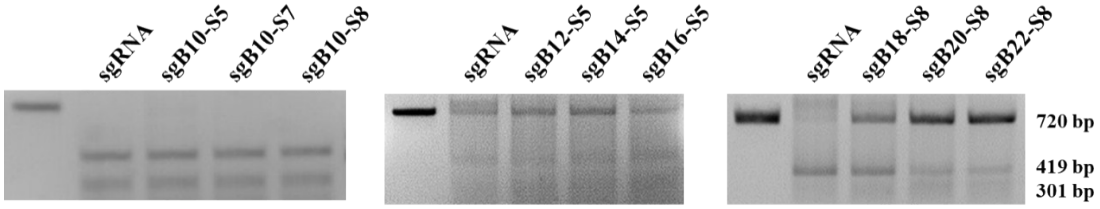

Figure S1. *In vitro* activity of unmodified sgRNA and sgBn-Sn sequences.

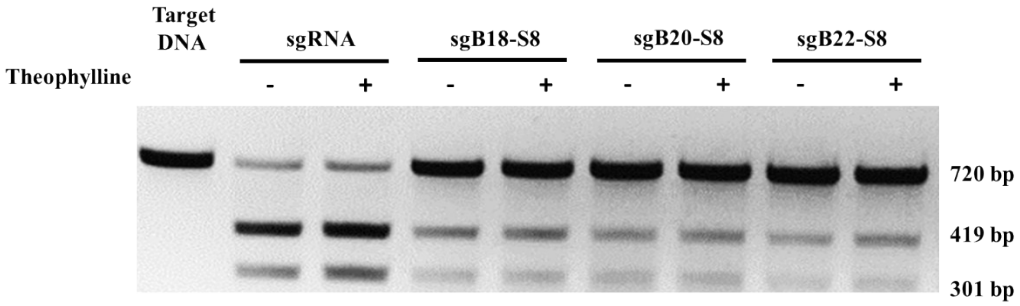

Figure S2. *In vitro* activity of unmodified sgRNA, sgBn-Sn sequences with or without theophylline (2 mM).

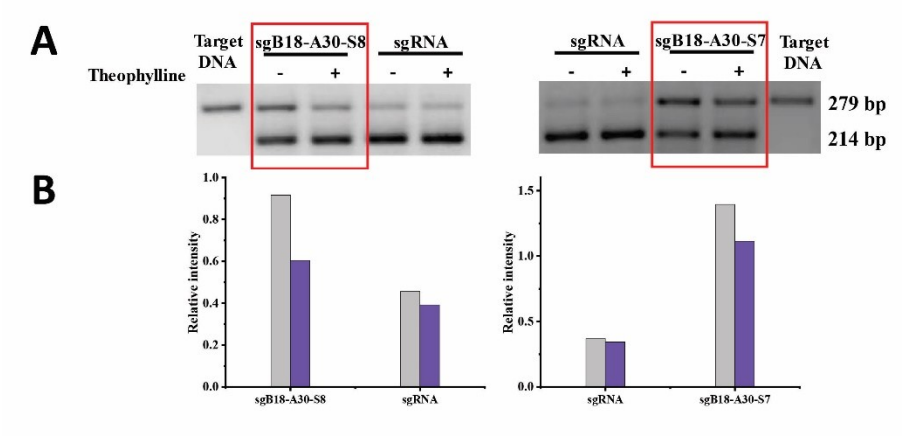

Figure S3. (A) TurboRFP DNA fragment cleaved by original sgRNA, sgB18-A30-S8 and sgB18-A30-S7 with or without theophylline (2 mM). The length of TurboRFP DNA fragment was 279 bp, and the cleavage products were 214 bp and 65 bp. (B) Intensity analysis of gel bands.

References

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