Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

A dual-functional microfluidic chip for guiding personalized lung cancer medicine: combining EGFR mutation detection and organoid-based drug response test

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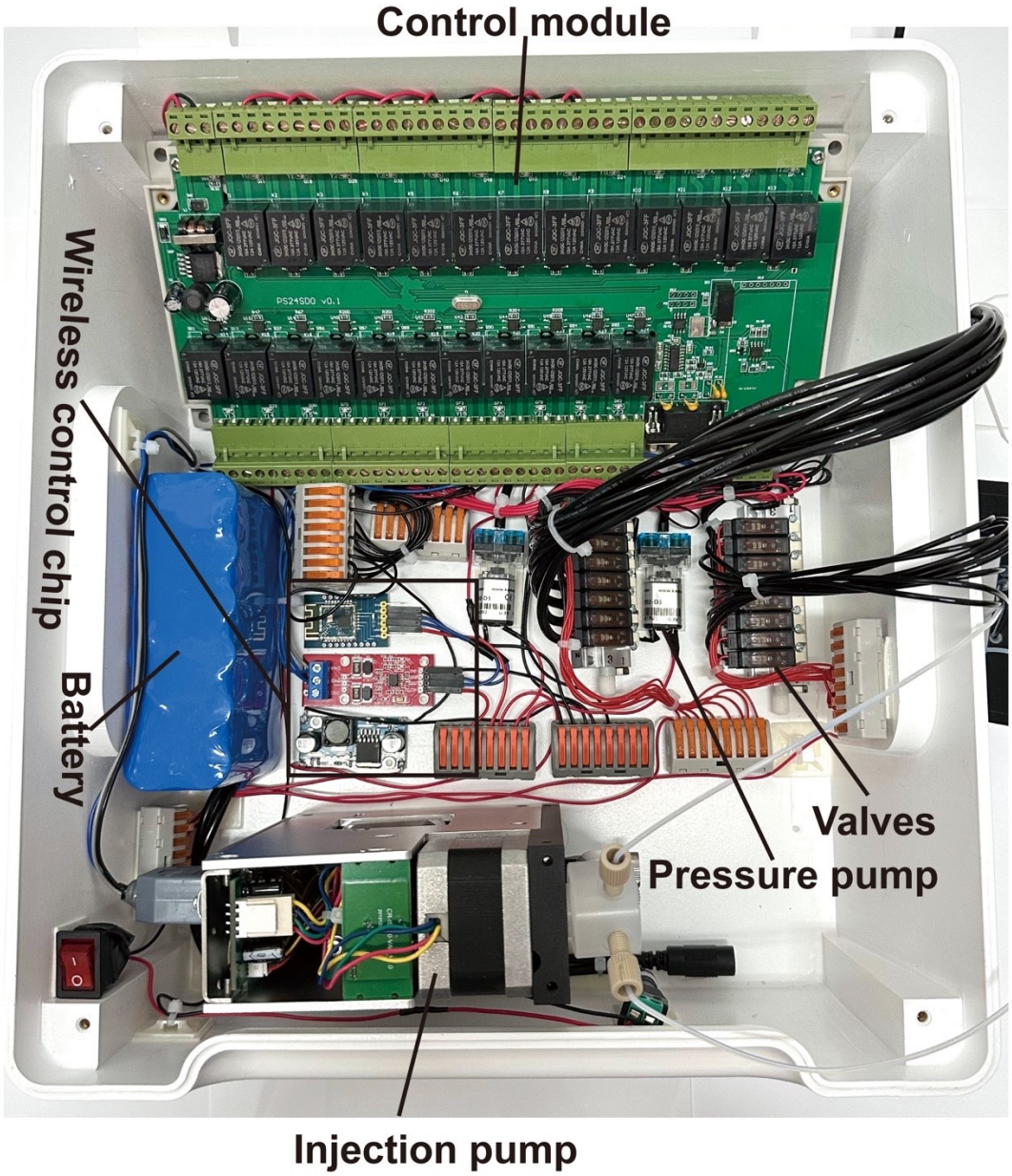

Fig. S1. Detailed interior structure of the RDMO-System. Associated control/communication modules consist of wireless communication & control circuits, valves, pumps and battery. In detail, the wireless communication & control circuit contains a Bluetooth chip, a RS232/RS485 chip, and a DC/DC chip. The 2 eight-channel solenoid valves controlled by pressure pump are used for facilitating liquid/gas transportation and regulating flow rates. The injection pump ensures the dynamic fluidic flow on the RDMO-System.

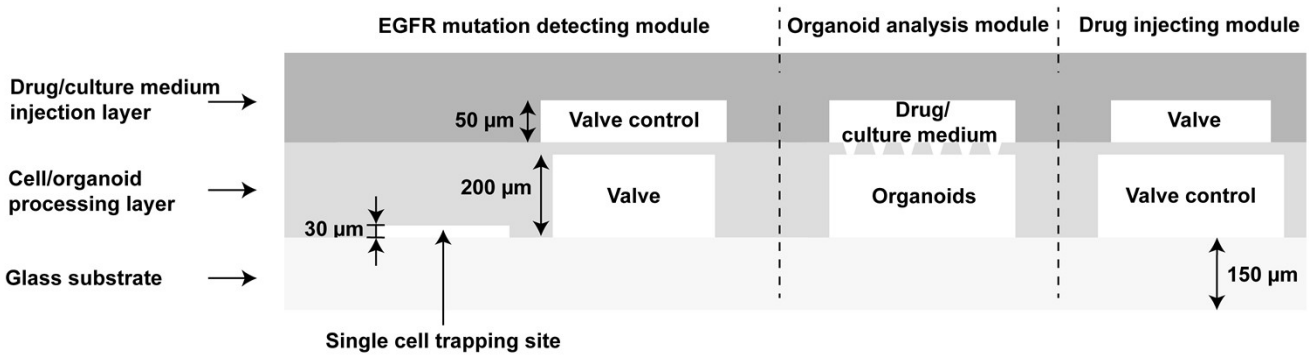

Fig. S2. The valve structure and vertical dimensions of RDMO-Chip. To fulfill the requirements of single cell trapping, the height of single cell trapping/staining channel is designed to 30 μm. To establish a sufficient space for organoid growth, the height of the organoid culture chamber is set to 200 μm. The height of the drug/culture medium channel is set to 50 μm. As there is no spatial overlap between valve area and function area for processing cells/organoids, no extra valve control layer is employed. In EGFR mutation detecting module, the valve control chambers locate at drug/culture medium injection layer. In organoid analysis module and drug injecting module, the valve control chambers locate at cell/organoid processing layer.

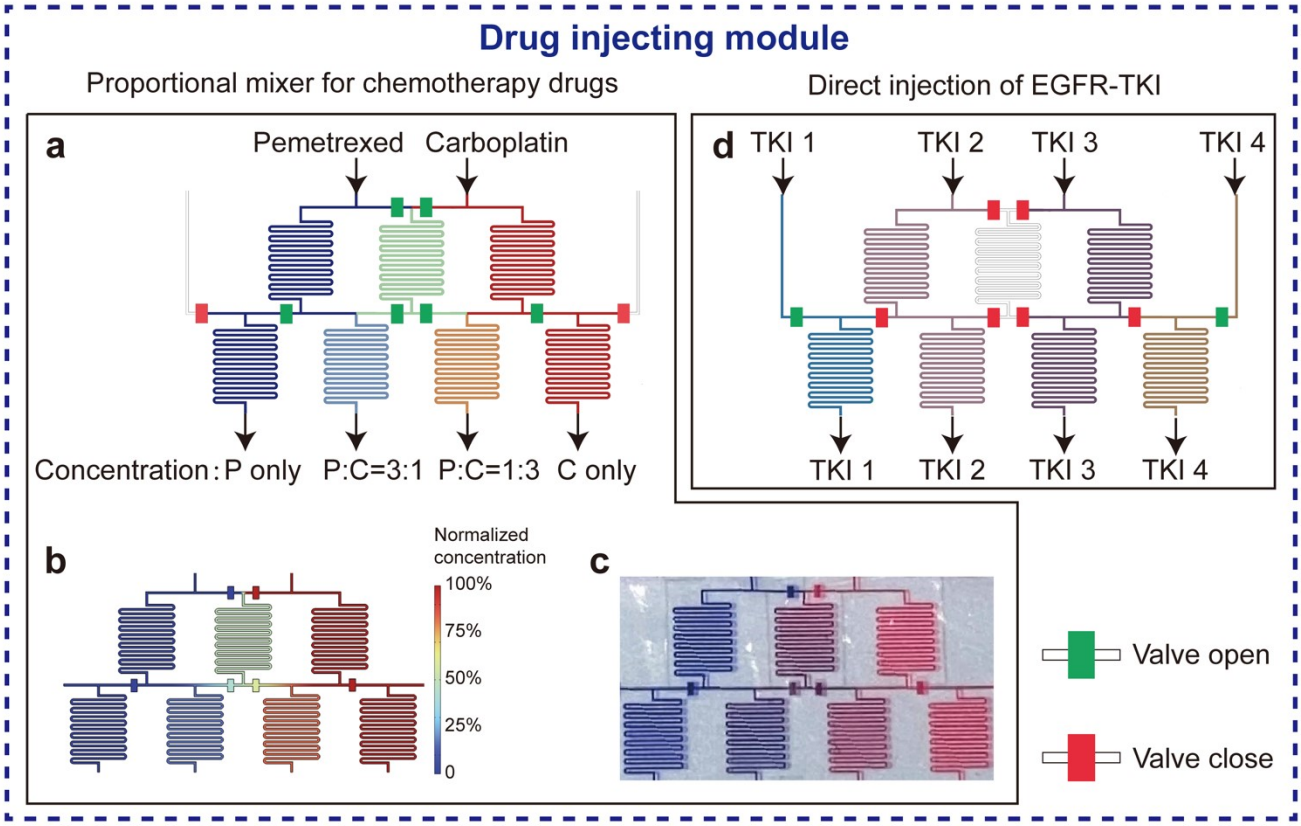

Fig. S3. The operations of drug injecting module. (a) The valve configuration of proportional mixer while administrating chemotherapy drugs. (b) The simulation of the mixing process of the proportional mixer. (c) The ink blending in the proportional mixer. (d) The valve configuration of proportional mixer while adding EGFR-TKIs. According to the latest NCCN guideline, EGFR-TKI should be used as a single agent while being considered as 1st-line medicine, no combined usage of different EGFR-TKIs is recommended. Under a configuration of proportional mixer while administrating chemotherapy drugs, pemetrexed and carboplatin were applied on PDOs with 4 different concentration ratios, matching a frequently-used clinical combination of pemetrexed and carboplatin; Under a configuration of proportional mixer while adding EGFR-TKIs, 4 kinds of EGFR-TKIs were independently directed to 4 organoid culture channels without mixing. In this work, 18 organoid chambers were divided into 6 channels. PDOs in 2 channels were without adding anti-cancer drugs, acting as a control group. PDOs in the rest 4 channels were exposed to anti-cancer drugs, acting as experimental groups. The configuration of both organoid chambers and proportional mixer can be easily adjusted according to different requirements.

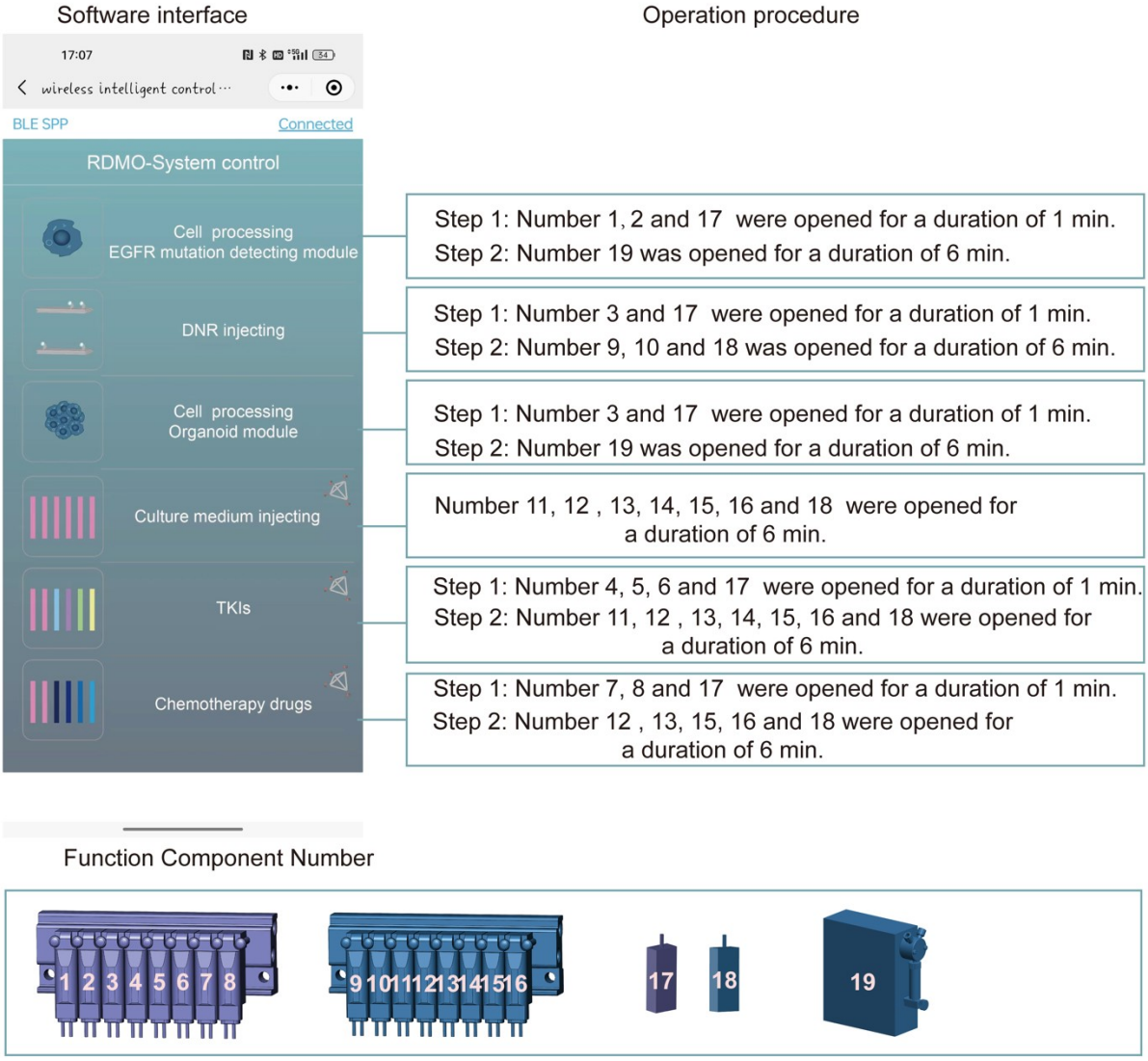

Fig. S4. Detailed operating procedures were initiated by using a specifically designed software running on a smartphone. The RDMO-System could be controlled using a specifically designed software running on a smartphone according to the therapeutic strategies and functional modes. The screenshot interface of the smartphone and operation procedure are displayed. No.1-8: eight-channel solenoid valves; No.9-16: eight-channel solenoid valves; No. 17/18: pressure pump; No. 19: control module. First, suspended cells dissociated from clinical samples were injected into the EDFR mutation detecting module for trapping single cell. Then, DNA-based nanorulers and cancer cells were simultaneously injected into the EDFR mutation detecting module and organoid analysis module, respectively, for cell staining of EGFR mutation detection and PDO culturing. EGFR mutation detection was accomplished during PDO culture. Finally, based on the result of EGFR mutation detection, PDO-based drug response test and monitoring were performed.

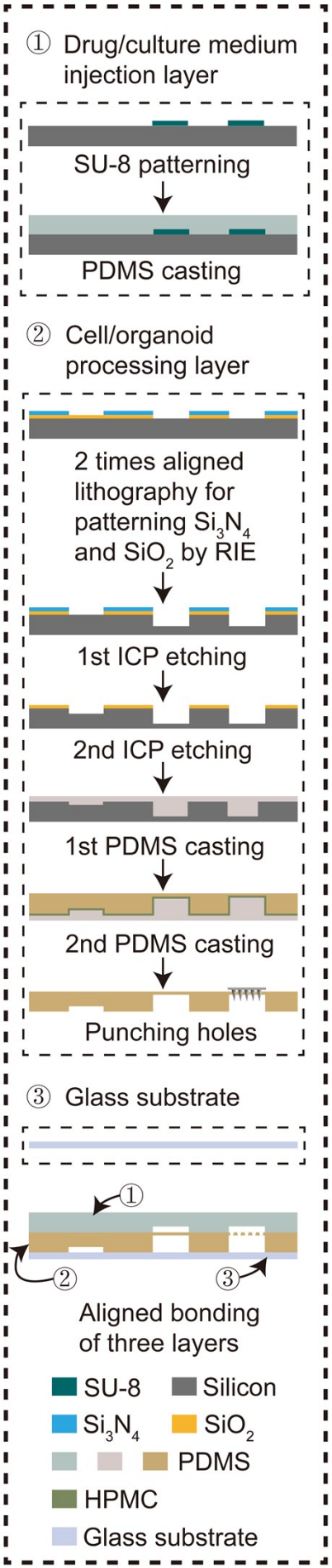

Fig. S5. The fabrication process of RDMO-Chip.

Briefly, 2 polydimethylsiloxane (PDMS) layers were separately fabricated and sequentially bonded to a glass substrate with alignment to form RDMO-Chip. The drug/culture medium injection layer was fabricated by casting PDMS onto a SU-8 mold which was coated and patterned on a 4-inch silicon wafer. The cell/organoid processing layer was formed by 2 times of PDMS casting. A silicon mold was fabricated by 2 times of inductively coupled plasma (dry) etching. As single cell processing and organoid culturing required different heights of microfluidic chambers, a SiO_2/Si_3N_4 mask for inductively coupled plasma (ICP) etching was formed by 2 times lithography with alignment and reactive ion etching (RIE).

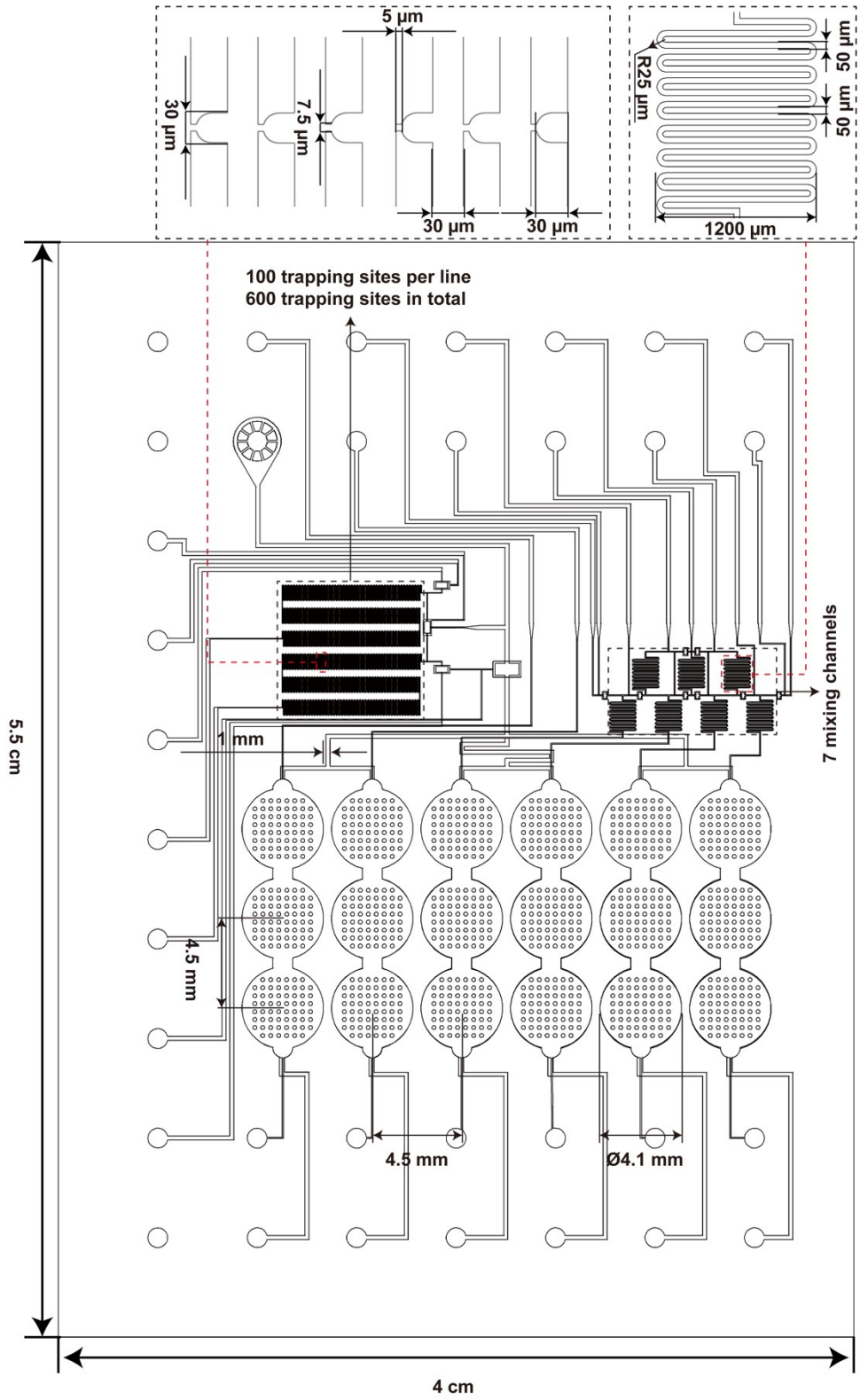

Fig. S6. Dimensions of RDMO-Chip. The dimensions of RDMO-Chip are marked on an overlapped layout. The single cell tapping channel is with 600 trapping sites (6 lines, 100 trapping sites per line). Upper left enlarged figure shows 6 single cell trapping sites with dimensioning. The proportional mixer consists of 7 identical mixing channels. Upper right enlarged figure shows a mixing channel with dimensioning.

Fig. S7. Route design of the three-layered rectangular structure. The M13mp18 scaffold ssDNA (blue) is folded into the three-layered rectangular structure by massive staple strands. The 9 special staple strands extended by polyA for connecting EGF molecules are designed on the three-layered rectangular structure. For EGFR-DNR-25, the distance between purple and green special strands is 72 bp≈25 nm. For EGFR-DNR-100, the distance between purple and red special strands is 296 bp≈100 nm.

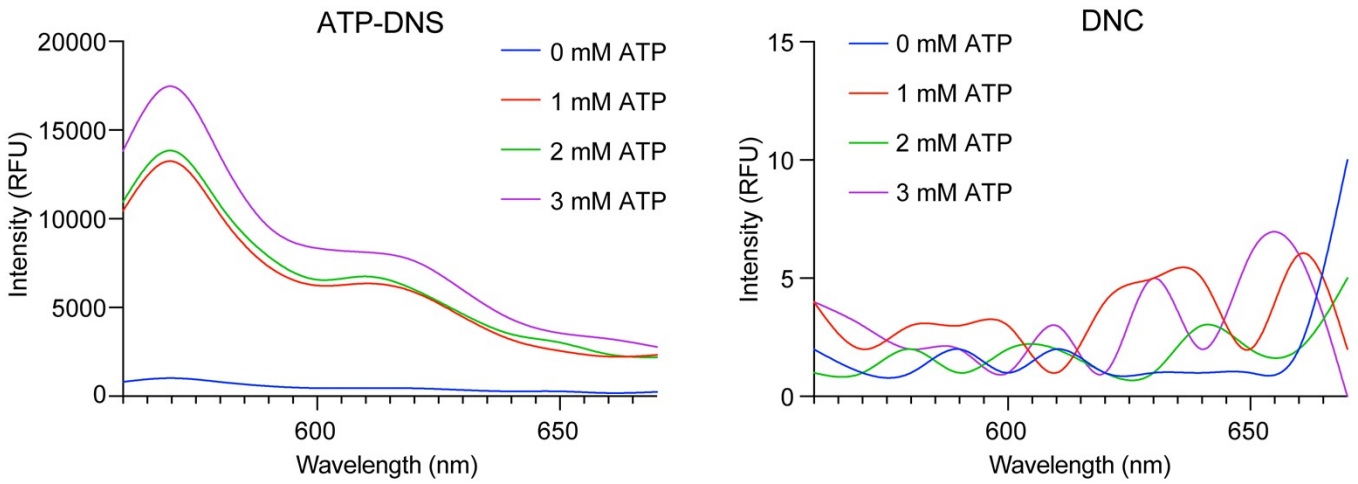

Fig. S8. Diverse fluorescence spectra of ATP-DNS and DNC to the response of ATP. Followed by the addition of 1-3 mM ATP respectively, sequential increases in fluorescence intensity of ATP-DNS are observed on 570 nm emission wavelength while DNC shows irregular fluorescence spectra. In addition, the fluorescence spectra of ATP-DNS and DNC show irregularity without ATP (0 mM ATP). These results demonstrate that ATP-DNS is sensitive to the response of ATP. The value of the fluorescence intensity of ATP-DNS excited is enhanced with the increase of ATP. As a negative control, DNC is incapable of responding to ATP.

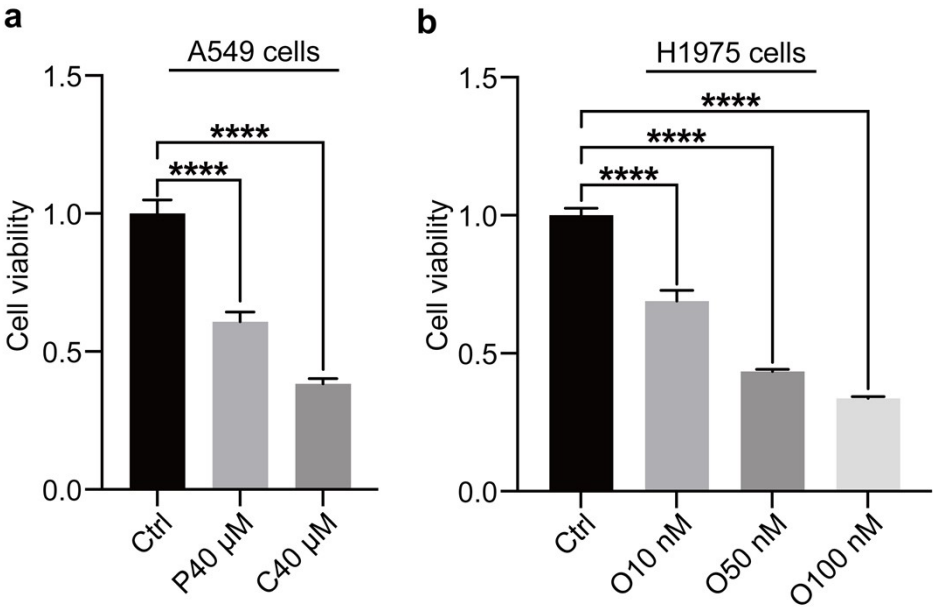

Fig. S9. The cell viability data of A549 and H1975 cells treated with diverse drugs. (a) The cell viability of A549 cells detected by CCK-8 under the 40 μM of pemetrexed (showed P) or carboplatin (showed C) at hour 48. (b) The cell viability of H1975 cells detected by CCK-8 under the different concentrations of osimertinib (showed O) at hour 48. The results illustrate that cell viability is changing with the treatment of the drug types or concentrations, showing similar consistency with the variation of ATP detected by ATP detection kits and ATP-DNS.

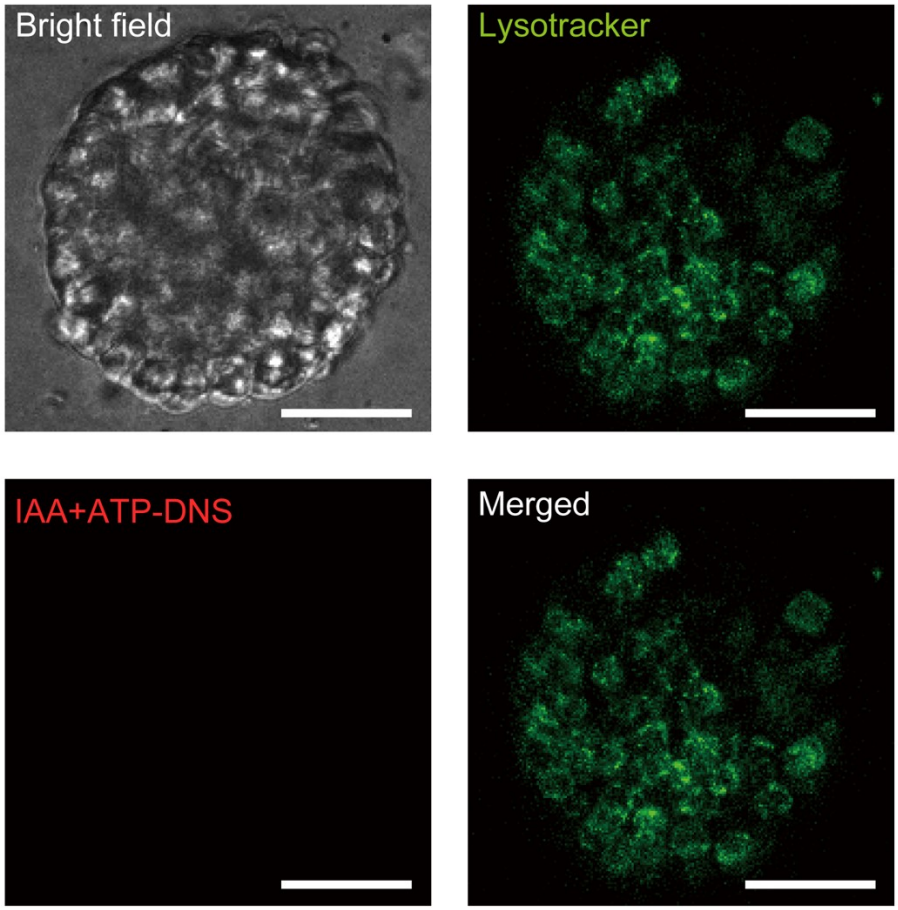

Fig. S10. Fluorescent images of PDOs treated with IAA+ATP-DNS (colored red) and stained with Lyso-Tracker (colored green). Before being treated with ATP-DNS, PDOs were pretreated with IAA (100 μM) for 4 h to decrease ATP generation. The intracellular locations were stained by Lyso-Tracker. Confocal images show that no red fluorescent signal was excited, demonstrating that ATP-DNS has high specificity for recognizing ATP. Scale bars: 50 μm.

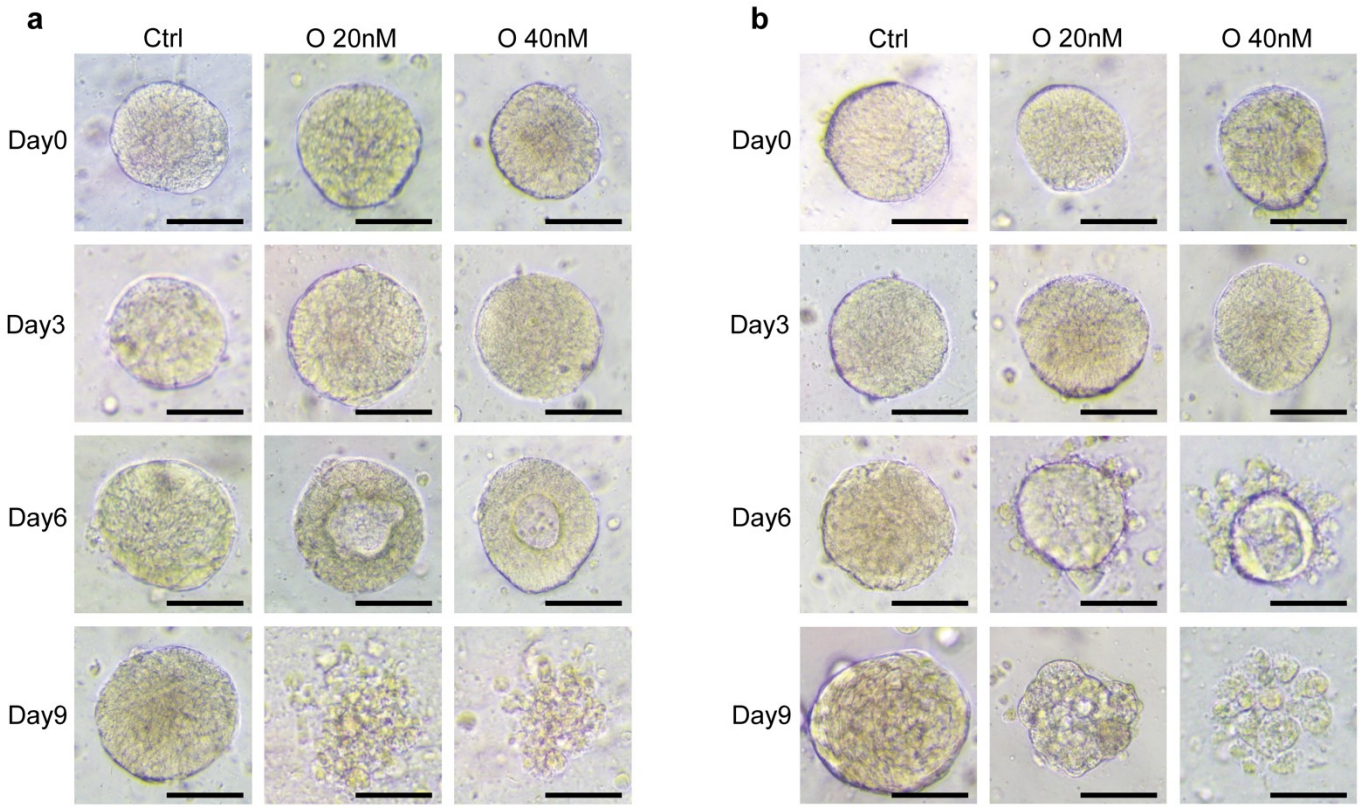

Fig. S11. The morphology of the PDOs treated by osimertinib. Organoids derived from (a) clinical sample 1 and (b) clinical sample 2 treated with osimertinib (20 nM) and osimertinib (40 nM) for 9 days. These bright images were captured every 3 days. The results illustrate that the PDOs from 2 clinical samples were inhibited with the increasing days, and showed no observable difference between groups treated with osimertinib (20 nM) and osimertinib (40 nM). O 20nM, osimertinib (20 nM). O 40nM, osimertinib (40 nM). Scale bars: 50 μm.

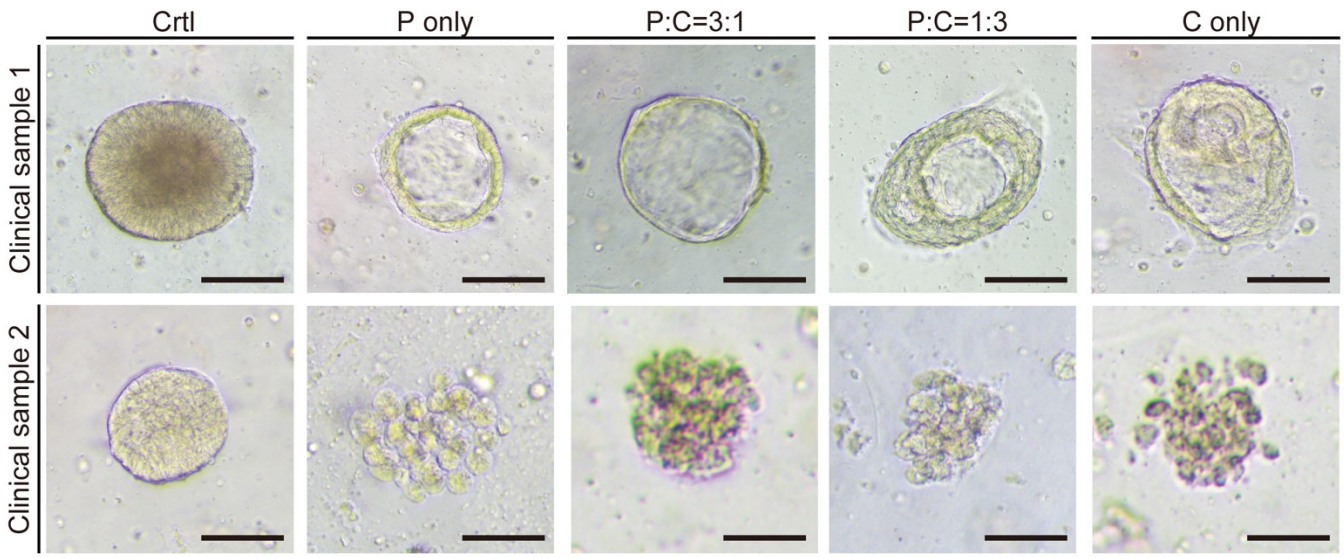

Fig. S12. The morphology of the PDOs treated by chemotherapy drugs. Organoids derived from 2 clinical samples were treated with different combinations of pemetrexed and carboplatin. These bright images were captured at day 6. The images demonstrate that there is no observable difference among these diverse chemotherapy treatments. P only, pemetrexed at 16 μM. P:C=3:1, pemetrexed at 12 μM and carboplatin at 4 μM. P:C=1:3, pemetrexed at 4 μM and carboplatin at 12 μM. C only, carboplatin at 16 μM. Scale bars: 50 μm.

Table S1. Detailed sequences of EGFR-DNR.

Staple strands	Sequence (5'-3')
Strand 1	AACGGGTATTAAACCAAGTACAAATAATACGTTAAATTGTAGCGGGAGCACGTATAAC
Strand 2	TTGTAGCGTAAATGCTTTAAAATATTCCAGGAAGAAAT
Strand 3	CGGAACGAGGGTACAACTTTCTGTTTAGAAAAAATCAGGTCACGTATCGATGA
Strand 4	CGGAGTGAGAATACAGCAGCGGCTCATTAATTCATTAGCGGGGAGAGGCGGTT
Strand 5	GATGATACAGGAGTGTCAAATGGTAGTTTGACCTATCAGGTGC
Strand 6	TGCGTATTTCCTGTGTAGGACGTTTTTTGCAACAACTAAAAACCCATG
Strand 7	TAATTGCGTTGCGAACGAGTA
Strand 8	CCGATAGTTGCGCATCAGCTTGCCAAAAGAAGCAAACGGAACGCCAATTCGCA
Strand 9	CCACCCTCAGAGCCACATAGATAAAATACCAATCACACACGACCAGTA
Strand 10	CAGTATTAACACCGCCACAGAGGTTCCTGATTATTAATTTTACCAGAAAGTAAGCA
Strand 11	CGGTCCACAAAACGACACATTCAACTAATCTAACAGCTTGGAG
Strand 12	CAAAGGGCCCTAAAGGTAGGGCTTTTTATCAAAAATAGCACCCAAAAG
Strand 13	AGCAATACTCCATCACGATAGCTTATGTAATTATCAAGATTTGCGGGAGGTTTTGA
Strand 14	TGAATCTTACCAAAAACAATGAATCATAGTCAATATAACTCAAACAAAACATC
Strand 15	GAATTTCTTAACATAAAAACAGTTAATTTCAGAGGCGCAGATTCACCAGTGCT
Strand 16	TAGGGTTGGGAAGAAAAAAGCCTGCTATATGTCTAATATCATACATAA
Strand 17	AGAGCCTAATTTGAGAATTGATCCGGCTTAATTACCTGAACAATATATTAGTC
Strand 18	CACCAGTGAGCTCGAAAACGAACTAGACGACGACGTTGAACCCTCATT
Strand 19	AGAACAAGCAAGCCGTTTTTATTTATCAATACTAGAAGCGAAAGGCAGAGCGGGAGCT
Strand 20	ATAAAAGGACAACTAAAGGATTTAGAAGTATTGCCACCCTAAAGGGCG
Strand 21	AAACAGGATGCAATGCGGCAAAGAATTAGCAACAGGTCAGGCGGGGTT
Strand 22	ACCCTTCTGAAGGTTATCGACAACTCGTATTAGAGCCACCAGGTAAAT
Strand 23	AGGAAACGAGGCCGGATATACTTCTTATCAGATGCCACGCTGAGAGCC
Strand 24	AAAACACTCATCTTTGACCCCAACCGGATTACCAGTCGAAATTGTCATTCAGGCTGCG
Strand 25	AAGTCAGAGGGTACCCAATCCATCATAATCAATAGATAAAGAATAGCCCGAGA
Strand 26	TGACAGGAGGTTGAGGAATTAAGCGGCTTAGATAAGCAAAGCCAGCTT
Strand 27	TGTACCCCCAAAAGGGTAGCATTAACATCCAACAAATAAAGCTGAGAC
Strand 28	GCCATTAAATGAAAAAGCGGAATTATCATCATTTAGCGTCACCATTAC
Strand 29	GGATTTTGCTAAAGCAACGGCTTGTGAATTCATTCAGCGTGCCAGCTGCATTA
Strand 30	GCGCATCGGCTGTCTTTCCTTAGAAACCAGGTTTGAACCGCCGCGCTTAACAC
Strand 31	GGAATCATTACCGCGCCCAATACAACATGTTATACAACGGCGAACGGGATTTTAGACA
Strand 32	AGTCCACTGGGAAAGCATTCTTACTTTTAACCGTTAAGCCTTACGCAG
Strand 33	AGGTGGCAAAATTATTCGTCAGATGCCCGAACAATCAACAGTTGAAAG
Strand 34	TACCGTTCCAGTAAGCCATTTGGGGTTGATTCACAAGAGATGGTGTAG

Strand 35	AGGCTTGCAGGGAATTTTTTCATAAAAACAGAGGAAGTGAGCGAGAGCCCCAA
Strand 36	TCACCGCCGCAGGTCGGAAAGATTTAAGAGCAAGGAGCCTCACCCTCA
Strand 37	TAATAAGAGCAAGCGCTAACGAGCCAACGATTCTGTCACGTGGACTCCAACGT
Strand 38	TCTGAAACCACTGAGTATTATAGTTTGCCAGACCATTCGCTATCCGCT
Strand 39	CGCCTCCCTCAGAGCCAGACTTTAAGAAACAAGGCCAACATCTGAAAT
Strand 40	AGAATCAAGTTTGCCTATTCCTGATGAATAATAACGAACCAACATCAC
Strand 41	TATGTTAGACCGACTTAATAAAGAAACATTATCAAACCCTCAATCAAT
Strand 42	TTGTAAACTAAAAATTCTCAGAGCATAAAGCTGAGCCGCCGCCGTCGA
Strand 43	GAGGGTTGCCACCCTCCAGACCGGGAATTACGGCGATTAACCAAGCTT
Strand 44	ACGGTAATTCACCATCTGAAAAGGTGGCATCAGCGCAGTCCCTATTAT
Strand 45	AGAGTCTGTTCTTTGACGCTATTAGTTTGGATAACGTCACGTAGCGAC
Strand 46	CCATCGATAACAAAGTTCCCTTAGAATCC
Strand 47	AACCTAAAACGAAAGAGGCAAAAAGCTGCTACCTTATATACGAGCTGGTGCCGGAAAC
Strand 48	ACTACAACCCTGCCTAATATAACAGCGCGAGCAATATGATATTCAACC
Strand 49	TGTCGTCTTTCCAGACGT
Strand 50	TAGACGGGAGAATACGTCAAAAAATAAGGTCCCATCCGTTCCGAAATCGGCAA
Strand 51	TTCAGGGAAGAAGGATTAATGCTGACAGGCAACTGAGTAATGTGTAGG
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0 nm special strand 2	TTGCCTGATAATCAGTATAGTGAAAATTGAGACAATTTTAAGA
0 nm special strand 3	ATCGGAACGAAAAACCAAAGTACCGACAAAAGGTATTCTATCC
0 nm-A special strand 1	AAAAAAAAAAAAAAAAGCAGCAAAAATACCGGGAAGGGTAATAACCTACGGAATAATA
0 nm-A special strand 2	AAAAAAAAAAAAAAAATTGCCTGATAATCAGTATAGTGAAAATTGAGACAATTTTAAGA
0 nm-A special strand 3	AAAAAAAAAAAAAAAAATCGGAACGAAAAACCAAAGTACCGACAAAAGGTATTCTATCC
25 nm special strand 1	ATCTGGTCAATATTTTAGATTTTCTTAACAATGAAAATACAGA
25 nm special strand 2	AGCCATTGCAGGAGGCTTATATAATTTAGTATAAAATAAAGTA
25 nm special strand 3	AAAGGAAGAGTGTTGTATGCAGAACGCGCCTGTTTTCATCCAG
25 nm-A special strand 1	AAAAAAAAAAAAAAAAATCTGGTCAATATTTTAGATTTTCTTAACAATGAAAATACAGA
25 nm-A special strand 2	AAAAAAAAAAAAAAAAAGCCATTGCAGGAGGCTTATATAATTTAGTATAAAATAAAGTA
25 nm-A special strand 3	AAAAAAAAAAAAAAAAAAAGGAAGAGTGTTGTATGCAGAACGCGCCTGTTTTCATCCAG
100 nm special strand 1	CAGTCAAACGTAAAACCTGGAAGTCCCTGACTTTCGTCACCAG
100 nm special strand 2	TGACCGTAGCAAAGCGGGGGGTAAAAGAACTGAAAGACAGACT
100 nm special strand 3	CACAATTCGCCAACGCCCCAAATCAACGTAACAAGAATACCAT
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100 nm-A special strand 2	AAAAAAAAAAAAAAAATGACCGTAGCAAAGCGGGGGGTAAAAGAACTGAAAGACAGACT
100 nm-A special strand 3	AAAAAAAAAAAAAAAACACAATTCGCCAACGCCCCAAATCAACGTAACAAGAATACCAT
PolyT	TTTTTTTTTTTTTTT-SH-C6

Table S2. Detailed sequences of ATP-DNS.

Strands	Sequence (5'-3')
CY3-modified ATP aptamer strands	CY3-CAGTCACCTGGGGGAGTATTGCGGAGGAAGGTAAAAAAAAAAAAAAA
BHQ2-modified short strands	CCCAGGTGACTG-BHQ2
BHQ2-modified long strands	GCAATACTCCCCCAGGTGACTG-BHQ2
ssDNA1	TTTTTTTTTTTTTTTAAT ACA TTC CTA AGT CTG AAACAT TAC AGC TTG CTA CAC GAG AAG AGC CGC CAT AGT A
ssDNA2	TTTTTTTTTTTTTTTAAT TAT CAC CAG GCA GTT GAC AGT GTA GCA AGC TGT AAT AGA TGC GAG GGT CCA ATA C
ssDNA3	TTTTTTTTTTTTTTTTTA TCA ACT GCC TGG TGA TAA AAC GAC ACT ACG TGG GAATCT ACT ATG GCG GCT CTT C
ssDNA4	TTTTTTTTTTTTTTTTTA TTC AGA CTT AGG AAT GTG CTT CCC ACG TAG TGT CGT TTG TAT TGG ACC CTC GCA T

A tetrahedral DNA framework was assembled with ssDNA1, ssDNA2, ssDNA3, and ssDNA4.

ATP-DNS was assembled with tetrahedral DNA framework and CY3-modified ATP aptamer strands linked with BHQ2-modified short strands.

DNC was assembled with tetrahedral DNA framework and CY3-modified ATP aptamer strands linked with BHQ2-modified long strands.