Supporting Information

Aptamer-Modified DNA Tetrahedra-Gated Metal-Organic Framework Nanoparticle Carriers for Enhanced Chemotherapy or Photodynamic Therapy

*Pu Zhang†, Amit Fischer†, Yu Ouyang, Jianbang Wang, Yang Sung Sohn, Rachel Nechushtai, Eli Pikarsky, Chunhai Fan and Itamar Willner**

† These authors contributed equally to this work.

Dr. Pu Zhang, Amit Fischer, Dr. Yu Ouyang, Dr. Jianbang Wang, Prof. Itamar Willner
Institute of Chemistry, Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.
Dr. Yang Sung Sohn, Prof. Rachel Nechushtai
Institute of Life Science, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.
Prof. Eli Pikarsky
The Lautenberg Center for Immunology and Cancer Research, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.
Prof. Chunhai Fan
School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules, and National Center for Translational Medicine, Shanghai Jiao Tong University 200240, Shanghai, China
E-mail: itamar.willner@mail.huji.ac.il

EXPERIMENTAL SECTION

Materials and Instruments. 2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid sodium salt (HEPES), sodium chloride, magnesium chloride, doxorubicin hydrochloride (DOX), Rhodamine 6G, Zn(II)-protoporphyrin IX (Zn(II)-PPIX), N, N, N', N'-tetramethylethylenediamine (TEMED), acrylamide solution (40%), agarose, ammonium persulphate, dimethylformamide (DMF), dibenzocyclooctyne-sulfo-*N*-hydroxysuccinimidyl ester (DBCO-sulfo-NHS), sodium azide (NaN_3), sodium nitrite ($NaNO_2$), HCl (37%), *N*-methyl-2-pyrrolidone (NMP), zirconium oxychloride ($ZrOCl_2$), 2-aminoterephthalic acid and 1,3-diphenylisobenzofuran (DPBF) were bought from Sigma-Aldrich. DNA oligonucleotides were synthesized and purified at Integrated DNA Technologies Inc. (Coralville, IA). Di(acetoxymethyl ester)-6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (CDCHF-DA) and SYBR Gold nucleic acid gel stain was purchased from Invitrogen. Ultrapure water from NANOpure Diamond (Barnstead) source was applied throughout the whole experiments.

A Magellan XHR 400L scanning electron microscope (SEM) was employed to characterize the microcarriers. Fluorescence spectra was measured with a Cary Eclipse Fluorometer (Varian Inc.). The excitation of DOX and Rh 6G were excited at 480 and 420 nm. The emission of DOX and Rh 6G were measured at 590 and 560 nm. The excitation of Zn(II)-PPIX was excited at 420 nm. The concentrations of DNA oligonucleotides were monitored using a UV-2401PC (SHIMADAZU) spectrophotometer. The gel experiment was run on a Hoefer SE 600 electrophoresis unit.

The sequences of all nucleic acids used in this paper are composed of: (from 5' to 3')

ATP aptamer-functionalized tetrahedra:

S1-ATP: ACACTACGTCAGA A CAGCTTGCATCAC T GGTCACCAGAGTA AAA TGG AAG GAG GCG TTA TGA GGG GGT

S2: ACGAGCGAGTTGA T GTGATGCAAGCTG A ATGC GAG GGT CCT

S3: TCTGACGTAGTGTATGCACAGTGTAGTTAGGACCCTCGCAT

S4: TCAACTCGCTCGTTACTACACTGTGCAATACTCTGGTGACC

Anchor **(1)**: NH_2-$(CH_2)_6$-CCC AAT CGA ACC CCC

(1''): ACACTACGTCAGA A CAGCTTGCATCAC T GGTCACCAGAGTA AAA TGG AAG GAG GCG TTA TGA GGG GGT

VEGF aptamer-functionalized tetrahedra:

S1-VEGF: ACACTACGTCAGA A CAGCTTGCATCAC T GGTCACCAGAGTA AAA T GTG GGG GTG GAC GGG CCG GGT AGA

S2: ACGAGCGAGTTGA T GTGATGCAAGCTG A ATGC GAG GGT CCT

S3: TCTGACGTAGTGTATGCACAGTGTAGTTAGGACCCTCGCAT

S4: TCAACTCGCTCGTTACTACACTGTGCAATACTCTGGTGACC

Anchor **(2)**: NH_2-$(CH_2)_6$-TCTACCCGGCCC

(2''): ACACTACGTCAGA A CAGCTTGCATCAC T GGTCACCAGAGTA AAA T GTG GGG GTG GAC GGG CCG GGT AGA

Generation of DNA tetrahedra. The ATP aptamer-functionalized DNA tetrahedra depicted in Figure 1 (B), consisted of four sequences, were prepared as follows. A mixture of (S_1-ATP), (S_2), (S_3) and (S_4) (2 μM each) in HEPES buffer (10 mM, 20 mM $MgCl_2$, pH = 7.2) was annealed at 95 °C for 5 min, subsequently, cooled down to 4 °C, and allowed to equilibrate at 25 °C for 2 hours, yielding DNA tetrahedra. The VEGF aptamer-functionalized tetrahedra were synthesized by following the same procedure.

Synthesis of UiO-66 NMOFs. The preparation of NMOFs was according to the reported method.[S1] First, 50 mg of 2-azido-terephthalic acid and 21 mg of $ZrOCl_2$ were mixed together in DMF (4 mL). Then, 2 mL of acetic acid were added to the mixture that was heated in an oven at 90 °C for 18 hours. After that, the resulting NMOFs were centrifuged and washed with DMF, triethylamine/ethanol (1:20, V/V), and ethanol.

Preparation of DBCO-functionalized nucleic acid (1) or (2). To link the DBCO functional groups to nucleic acid **(1)** or **(2)** (DBCO = dibenzocyclooctyne), 200 μL of 1×10^{-4} M **(1)** or **(2)** was added to 20 μL of 5×10^{-2} M DBCO-sulfo-NHS (dissolved in water) and shake overnight. The obtained solution was filtered through a MicroSpin G-25 columns (GE-Heathcare) to remove excess DBCO.

Synthesis of DNA (1)- or (2)-functionalized NMOFs. Initially, NMOF nanoparticles (1 mg, 1 mL) were treated with DBCO-DNA **(1)** or **(2)** (100 nmol). Then, the resulting solution was diluted with NaCl to a final concentration of 0.5 M and incubated over 6 h. Subsequently, the resulted solution was stirred at 40 °C for 72 h (600 rpm). The DNA **(1)** or **(2)**-functionalized NMOFs were washed by water to remove unbound nucleic acids.

The preparation of drug/dye-loaded NMOFs. To load the drug or dye, 1 mg of DNA **(1)**- or **(2)**-, **(4)**-functionalized NMOFs were shake with doxorubicin, DOX (1 mM) or Rh 6G (0.5 mM) or Zn(II)-PPIX (0.01 mM) for 12 h in 1 mL water. The DNA **(1)**- or **(2)**- functionalized NMOFs were then transferred to a buffer solution and hybridized with the ATP aptamer-functionalized DNA tetrahedra or VEGF aptamer-functionalized DNA tetrahedra, or the control system of nucleic acid **(1'')** or **(2'')**, respectively, resulting in the locked state of the NMOFs encapsulated the respective drugs/dyes. 12 hours later, the NMOFs were washed several times to remove the unloaded drug/dye. The drug/dye-loaded tetrahedra-gated or duplex-gated NMOFs were kept at 4 ^{o}C for further use.

ATP-responsive unlocking of the tetrahedra-gated NMOFs and the release of the loads. The ATP-responsive loaded NMOFs, 0.1 mg, were subjected to 1mL buffer solutions containing different concentrations of ATP or in the absence of ATP. At appropriate time intervals, samples of the mixture are centrifuged to precipitate the NMOFs (10 000 rpm for 2 minutes). The fluorescence of the released loads in the supernatant solution was measured

using a Cary Eclipse Fluorescence Spectro- photometer (Varian Inc.).

VEGF-responsive unlocking of the tetrahedra-gated NMOFs and the release of the loads. The VEGF-responsive loaded NMOFs, 0.1 mg, were subjected to 1mL buffer solutions containing different concentrations of VEGF or in the absence of VEGF. At appropriate time intervals, samples of the mixture are centrifuged to precipitate the NMOFs (10 000 rpm for 2 minutes). The fluorescence of the released loads in the supernatant solution was measured using a Cary Eclipse Fluorescence Spectro- photometer (Varian Inc.).

AFM Imaging. For the AFM experiment, the freshly peeled mica was modified with 0.5% APTES for 30sec and then washed by deionized water and dried with nitrogen. Then 5 μL, 10 nM of prepared DNA tetrahedra samples were deposited on the mica surfaces. After adsorbing for 10 min, the samples were imaged using the tapping mode in an aqueous buffer using SNL-10 probes (Bruker, Multimode Nanoscope VIII).

ROS detection by DPBF. 0.1 mg of Zn(II)-PPIX-loaded VEGF-responsive tetrahedra-gated NMOFs was treated with 2 μM of VEGF for three hours and the NMOFs were centrifuged and the washing solution was collected. The collected solution was subjected to 30 μM DPBF and was illuminated for different time-intervals at λ= 405 nm, 30 mW/cm^2. The resulted solution was measured by UV-2401PC (SHIMADAZU) spectrophotometer.

Cell culture. Normal breast cells (MCF-10A), non-tumorigenic human breast epithelial cell line, were maintained in complete growth medium consisting of 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium supplemented with horse serum (5%), epidermal growth factor (20 ng/mL), cholera toxin (CT, 0.1 μg/mg), insulin (10 μg/mL), hydrocortisone (500 ng/mL), and penicillin/streptomycin (1 unit/mL). Human breast cancer cells (MDA-MB-231), human breast cancer cell line, were grown in 5% CO_2 RPMI-1640 medium supplemented with 10% FCS, L-glutamine, and antibiotics (Biological Industries).

Cells were plated one day prior to the experiment on 96-well plates for cell viability or on μ-slide 4 well glass bottom (ibidi) for confocal microscopy.

Cell viability experiments. Cytotoxicity was measured after incubation of the duplex-gated DOX-loaded NMOFs or the tetrahedra-gated DOX-loaded NMOFs, Zn(II)-PPIX-loaded VEGF-responsive duplex-gated NMOFs or Zn(II)-PPIX-loaded VEGF-responsive tetrahedra-gated NMOFs in MDA-MB-231, MCF-10A cells planted at a density of 1.2×10^4 cells per well in 96-well plates. After 6 hours incubation with the NMOFs, 60 μg/mL, cells were washed intensively with growth medium. And then cells were further incubated with the full cell medium for 3 days and the cytotoxicity was determined after 3 days with the fluorescent redox probe, Presto-Blue. The fluorescence of Presto-Blue was recorded on a plate-reader (Tecan Safire) after 1 h of incubation at 37 °C (λex = 560 nm; λem = 590 nm).

Confocal microscopy measurements. Cells, 2×10^5, were planted in μ-slide 4 well glass bottom on one day prior to the experiment. Cells were incubated with the duplex-gated DOX-loaded NMOFs or the tetrahedra-gated DOX-loaded NMOFs, Zn(II)-PPIX-loaded VEGF-responsive duplex-gated NMOFs or Zn(II)-PPIX-loaded VEGF-responsive tetrahedra-gated NMOFs (60 μg/mL of each sample) for 6 hours and then washed with DMEM-HEPES twice. DOX fluorescence in cells was monitored with the confocal microscopy (the Olympus FV3000 confocal laser-scanning microscope) and all images were analyzed with image J.

ROS production. ROS production in cancer cells was determined by incubating cells, 2×10^5, loaded with Zn(II)-PPIX-loaded VEGF-responsive duplex-gated NMOFs or Zn(II)-PPIX-loaded VEGF-responsive tetrahedra-gated NMOFs, 60 μg/mL, at 37°C with 10 μM di(acetoxymethyl ester)-6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (CDCHF-DA) in HEPES-buffered saline (HBS) supplemented with 10 mM glucose after the exposure of cells to the visible light (λ= 405 nm for 15 minutes, 30 mW/cm^2). This non-fluorescent molecule is

readily converted to a green-fluorescent form when the acetate groups are removed by intracellular esterases and oxidation by the activity of ROS within the cells. The conversion of the non-fluorescent indicator to the green fluorescent indicator was measured on line for 1h at 37°C under the confocal microscopy (the Olympus FV3000 confocal laser-scanning microscope) (λ_{ex} = 488 nm; λ_{em} = 517 nm) and all images were analyzed with image J.

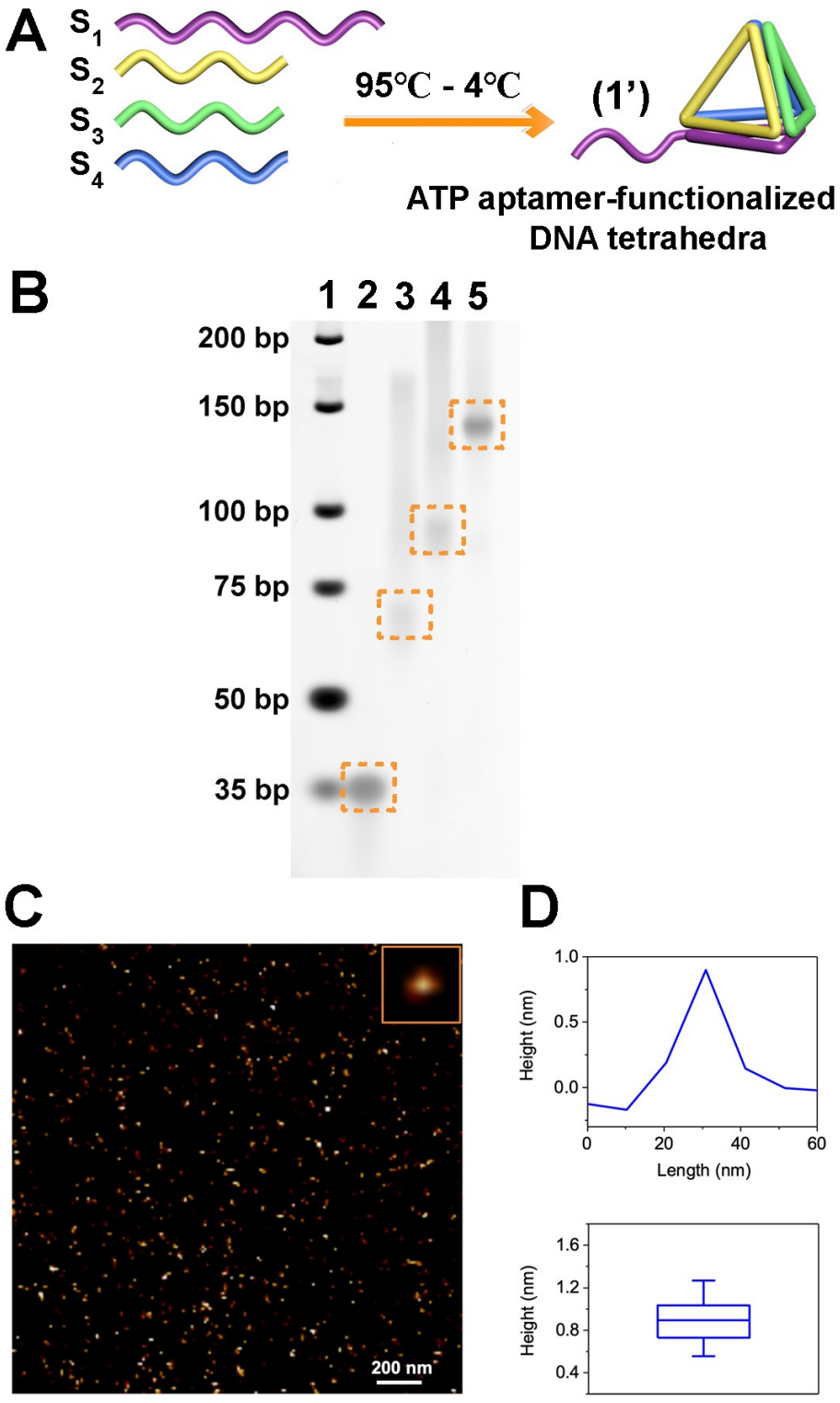

Figure S1. (A) Schematic assembly of the ATP aptamer-functionalized DNA tetrahedra using the strands S_1-S_4 (for detailed sequences see experimental section). The tether **(1')** is complementary to strand **(1)**-modified NMOFs and used to link the tetrahedra units to the NMOFs, through hybridization. (B) Electrophoretic separation following the stepwise

assembly of the tetrahedra structure. Electrophoresis on 12% PAGE gel: Lane 1-Ladder; Lane 2-S_1; Lane 3-S_1+S_2; Lane 4-S_1+S_2+S_3; Lane 5-S_1+S_2+S_3+S_4. (C) AFM image corresponding to the ATP aptamer-functionalized DNA tetrahedra. Insets correspond to enlarged structure of the tetrahedra. (D) Cross-section analysis of the height corresponding to the ATP aptamer-functionalized DNA tetrahedra.

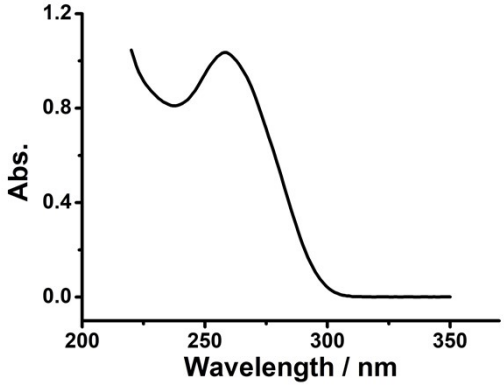

Figure S2. Evaluation of the loading of DNA anchor **(1)** associated with the NMOFs. 2 mg of NMOFs were introduced into a solution of 2 mL that contained 200 nmols of DNA anchor **(1).** The absorption spectrum of the solution was recorded prior to the addition of the NMOFs. After reaction of the NMOFs with DNA anchor **(1)**, the NMOFs were precipitated and the absorption spectrum of the supernatant was recorded to evaluated the concentration of unreacted DNA anchor **(1)**. The NMOFs were washed twice with water and the spectra of the washing solution were recorded, spectra (i) and (ii), respectively. The concentrations of **(1)** in the rinsing solution were added to the primary concentrations of non-reacted **(1)** and the total concentration of residual **(1)** subtracted from the initial concentration of **(1)**, reacted with the NMOFs to quantitatively evaluate the loading of **(1)** on the NMOFs. Using this procedure, the loading of **(1)** corresponded to 27.3 nmols per mg of NMOFs.

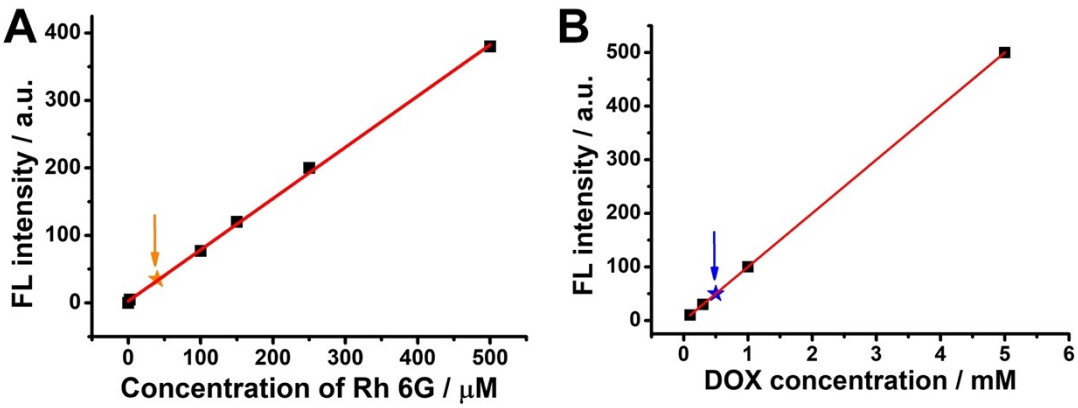

Figure S3. (A) The calibration curve corresponding to the fluorescence intensities as a function of the concentration of Rh 6G. The orange point is the evaluation of the loading of Rh 6G in the NMOFs. 0.1 mg of NMOFs were introduced in 1mL of a 0.5 mM Rh 6G solution. The mixture was stirred for 12 hours. Afterwards, the NMOFs were precipitated and the fluorescence spectrum of the supernatant solution was recorded, the pink point, and using the calibration curve, the loading of Rh 6G corresponded to 68 nmols per 1mg of NMOFs. (B) The calibration curve corresponding to the fluorescence intensities as a function of the concentration of DOX. The blue point is the evaluation of the loading of DOX in the NMOFs. 0.1 mg of NMOFs were introduced in 1mL of 1 mM DOX solution. The mixture was stirred for 12 hours. Afterwards, the NMOFs were precipitated and the fluorescence spectrum of the supernatant solution was recorded, and using the calibration curve, the loading of DOX corresponded to 65 nmols per 1mg of NMOFs.

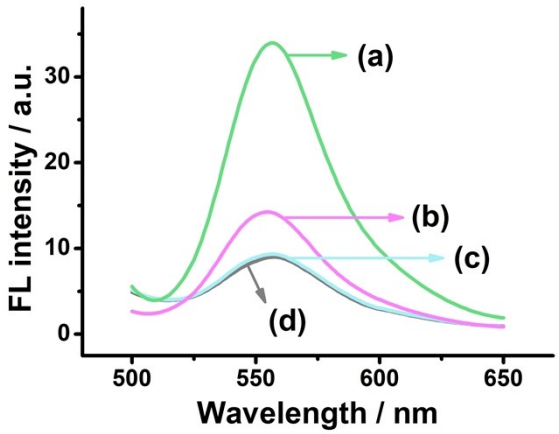

Figure S4. Fluorescence spectra of Rh 6G from the ATP-responsive tetrahedra-gated NMOFs in the presence of (a) 25 mM ATP; (b) 25 mM GTP; (c) 25 mM CTP and (d) 25 mM TTP.

Time-dependent release of Rh 6G from the ATP-responsive Rh 6G-loaded (1)/(1'')-gated NMOFs

In order to examine the superior features of the tetrahedra-functionalized drug-loaded NMOFs as carriers for the treatment of malignant cancer cells over drug-loaded NMOFs carriers gated by simple duplex DNA units, we synthesized Rh 6G-loaded duplex DNA-gated NMOFs presented in Figure S5 (A). In this system, the Rh 6G-loaded **(1)**-modified NMOFs were locked by strand **(1'')** through the hybridization of **(1)/(1'')**, generating **(1)/(1'')**-gated NMOFs. **(1)** is the anchoring strand associated with the NMOFs and the strand **(1'')** is

composed of the ATP-responsive sequence, ATP aptamer, complementary to **(1)**. Treatment of these control particles to ATP is anticipated to dissociate strand **(1'')**, due to the formation of the ATP-aptamer complexes, resulting in the release of Rh 6G. Figure S5 (A) depicts the ATP-stimulated release of the load. Effective release of the Rh 6G proceeds, similar to the release of Rh 6G from the tetrahedra-gated NMOFs. After *ca.* 250 minutes, the release was completed and the loading of Rh 6G in the NMOFs was very similar to the loading of the the tetrahedra-gated NMOFs. Thus, the *in vitro* experiments indicate very similar loading and release patterns of the drug integrated in **(1)/(1'')**-gated NMOFs and the tetrahedra-gated NMOFs. Nonetheless, as will be pointed later, *vide infra*, the permeation efficacy of tetrahedra-gated NMOFs into the malignant cells and their cytotoxic effect toward the cancer cells is significantly higher as compared to the **(1)/(1'')**-gated NMOFs, these demonstrating their enhanced anti-cancer functional capacity and cell permeation.

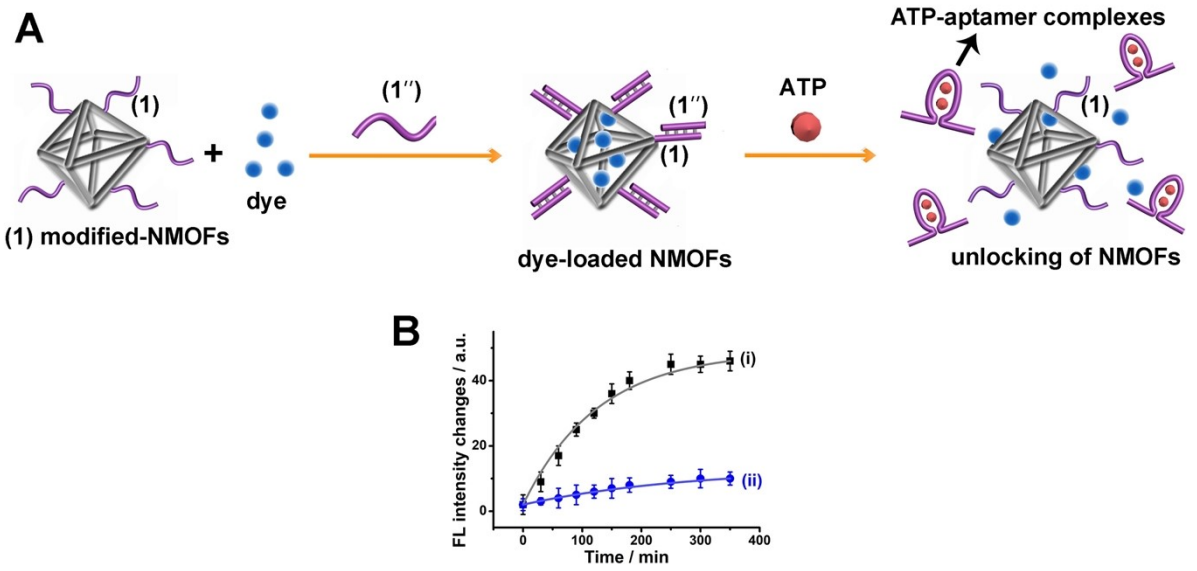

Figure S5. (A) Assembly of the Rh 6G-loaded (**1**)/(**1''**)-gated NMOFs and the ATP-induced release of the load, through the formation of the ATP-aptamer complexes. (B) Time-dependent release of Rh 6G from the Rh 6G-loaded (**1**)/(**1''**)-gated NMOFs: (i) in the presence of (i) 25 mM ATP and (ii) 0 mM ATP. Error bars derived from $N = 3$ experiments.

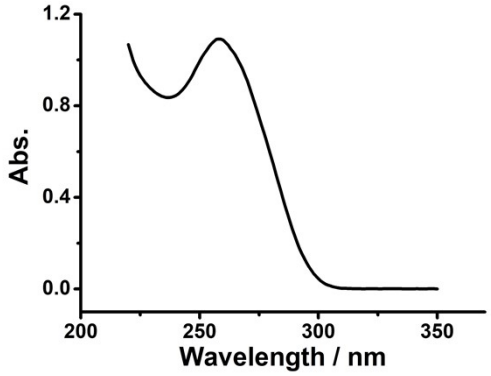

Figure S6. Evaluation of the loading of DNA anchor **(1)** associated with the NMOFs. The modification and evaluation of **(1)** follow the same procedure as before. Using this procedure, the loading of **(1)** corresponded to 27.1 nmols per mg of NMOFs. This result demonstrates that the coverage of the ATP-aptamer responsive functionalized NMOFs gated by duplex units were very similar to the DNA tetrahedra-gated carriers.

The incorporation of hemin into the K^+-ions-stabilized G-quadruplex yields the hemin/G-quadruplex DNAzyme that catalyzes the oxidation of Amplex Red by H_2O_2 to the fluorescent Resorufin product.

We note that the released VEGF-aptamer complexes reveal a G-quadruplex structure. This feature was confirmed by subjecting the released VEGF/tetrahedra structures to hemin to yield the respective catalytic hemin/G-quadruplex DNAzyme. The DNAzyme characteristic catalyzed oxidation of Amplex Red to the fluorescent Resorufin was shown in Figure S7. Curve (i) shows the time-dependent fluorescence changes of the fluorescent Resorufin upon subjecting VEGF/tetrahedra structures to K^+-ions. While subjecting the VEGF/tetrahedra structures in the absence of K^+-ions, curve (ii) or subjecting the NMOFs to K^+-ions in the absence of VEGF, curve (iii) show very inefficient fluoresce changes.

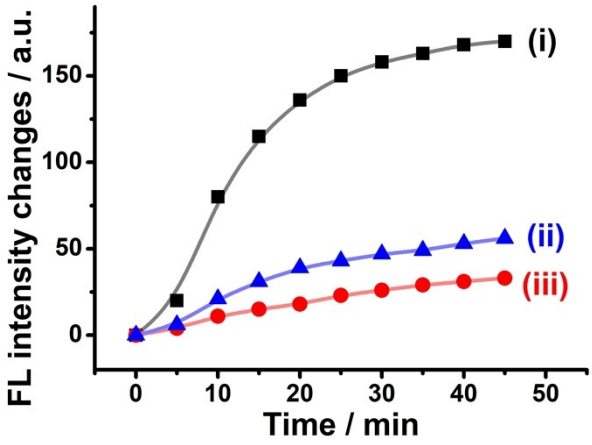

Figure S7. Time-dependent fluorescence changes of the fluorescent Resorufin upon: (i) Subjecting VEGF/tetrahedra structures to K^+-ions. (ii) Subjecting the VEGF/tetrahedra structures in the absence of K^+-ions. (iii) Subjecting the NMOFs to K^+-ions in the absence of VEGF.

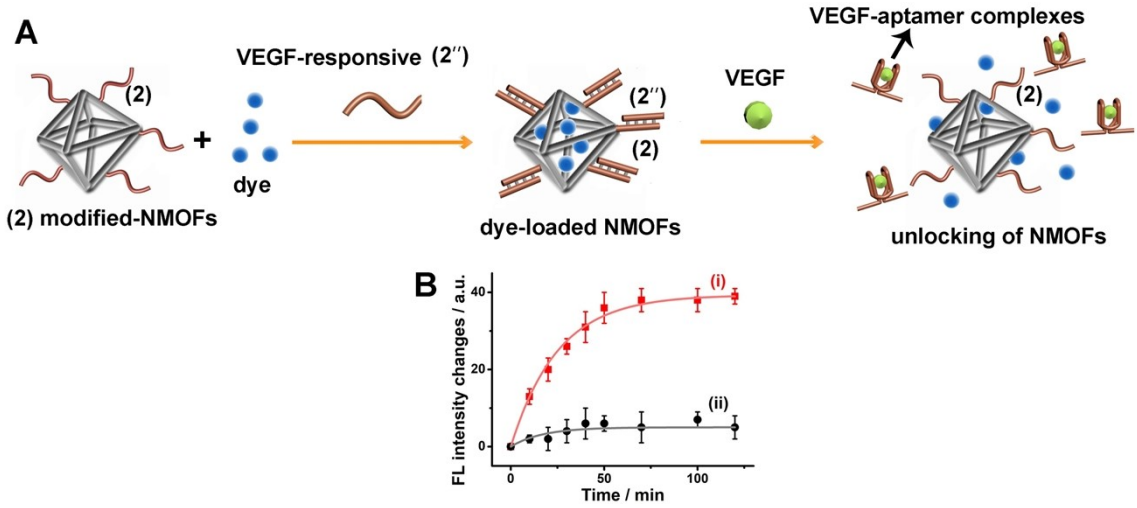

Figure S8. (A) Assembly of the Rh 6G-loaded (**2**)/(**2''**)-gated NMOFs and the VEGF-induced release of the load, through the formation of the VEGF-aptamer complexes. (B) Time-dependent release of Rh 6G from the Rh 6G-loaded (**2**)/(**2''**)-gated NMOFs: (i) in the presence of (i) 2 μM VEGF and (ii) 0 μM VEGF. Error bars derived from $N = 3$ experiments.

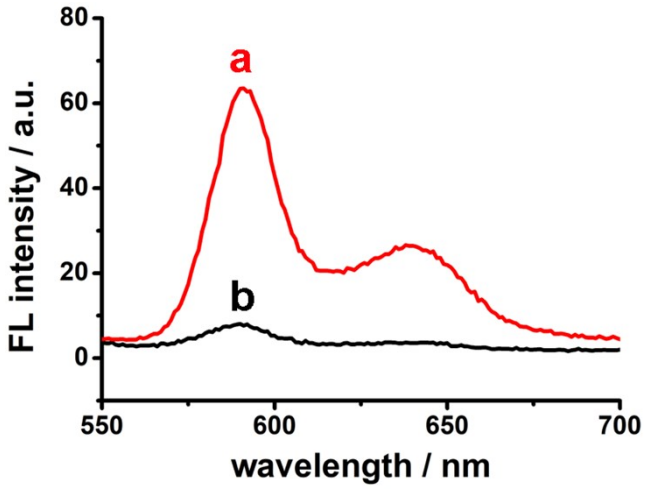

Figure S9. Fluorescence spectra of (a) 2 μM Zn(II)-PPIX associated with 2 μM G-quadruplex. (b) 2 μM Zn(II)-PPIX in water. It demonstrated that the fluorescence intensity of the Zn(II)-PPIX associated with the G-quadruplex tetrahedra structure is 8-fold higher as compared to the free Zn(II)-PPIX.

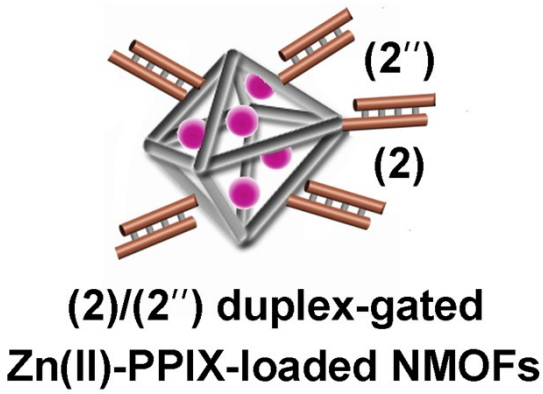

Figure S10. Schematic structure of the VEGF-responsive Zn(II)-PPIX-loaded NMOFs gated by the (**2**)/(**2''**) duplex-gated NMOFs.

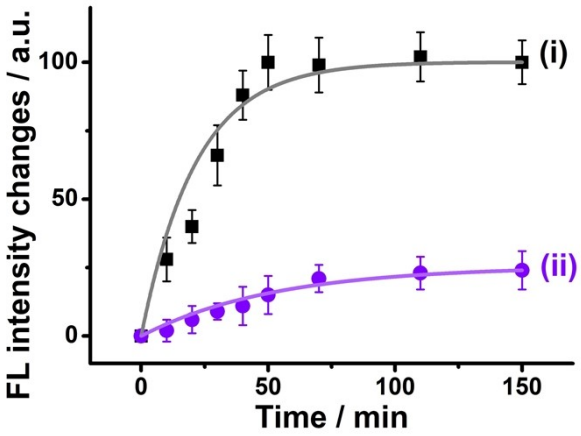

Figure S11. Time-dependent release and formation of the Zn(II)-PPIX/VEGF aptamer linked to the tetrahedra: (i) In the presence of VEGF, 2 μM. (ii) Subjecting the released Zn(II)-PPIX VEGF-aptamer G-quadruplex units to 200 mM 18-crown-6-ether. It is obvious that in the presence of added 18-crown-6-ether to the release mixture, the fluorescence intensities of the released Zn(II)-PPIX are substantially lower.

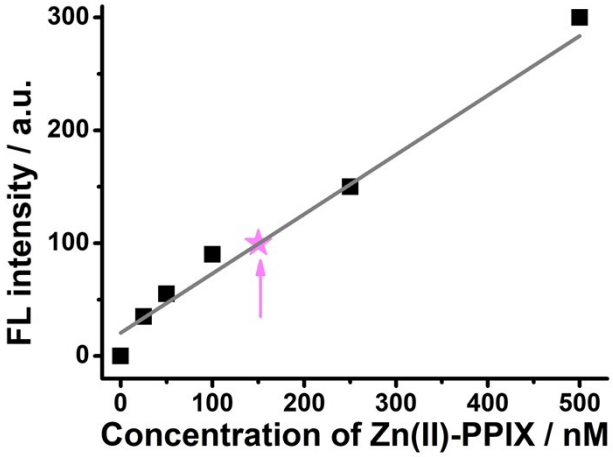

Figure S12. The calibration curve corresponding to the fluorescence intensities as a function of the concentration of Zn(II)-PPIX. The pink point is the evaluation of the loading of Zn(II)-PPIX in the NMOFs. 0.1 mg of NMOFs were introduced in 1 mL of a 0.01 mM Zn(II)-PPIX solution. The mixture was stirred for 12 hours. Afterwards, the NMOFs were precipitated and the fluorescence spectrum of the supernatant solution was recorded, the pink point, and using the calibration curve, the loading of Zn(II)-PPIX corresponded to 82 nmols per 1mg of NMOFs.

REFERENCES

S1 P. Zhang, Y. Ouyang, Y. S. Sohn, R. Nechushtai, E. Pikarsky, C. Fan, and I. Willner, *ACS Nano* **2021**, *15*, 6645-6657.