Electronic Supplementary Information

of

Targeted VEGF-Triggered Release of an Anti-Cancer Drug from Aptamer-Functionalized Metal-Organic Framework Nanoparticles

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Experimental Section

Materials: Vascular endothelial growth factor 165 human (VEGF), thrombin, hemoglobin, bovine serum albumin (BSA), Zirconium(IV) chloride ($ZrCl_4$), N',N'-dimethylformamide (DMF), trimethylamine, ethanol, tetrahydrofuran (THF), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid sodium salt (HEPES), tert-butyl nitrite (tBuONO), azidotrimethylsilane ($TMSN_3$), sodium chloride (NaCl), dibenzocyclooctyne-sulfo-N-hydroxysuccinimidyl ester (DBCO-sulfo-NHS), Rhodamine 6G, and doxorubicin were purchased from Sigma-Aldrich. Ultrapure water was obtained by a NANOpure Diamond instrument (Barnstead International, Dubuque, IA, USA). All nucleic acid strands were provided by Integrated DNA Technologies Inc. (Coralville, IA). The detailed DNA sequences used in the present study are:

(**1**) 5'-NH_2-$(CH_2)_6$-TCTACCCGGCCC-3';

(**2**) 5'-TGTGGGGGTGGACGGGCCGGGTAGA-3';

(**3**) 5'-GGTGGTGGTGGTTGTGGTGGTGGTGGTGTGGGGGTGGACGGGCCGG GTAGA-3'.

Synthesis of azide-functionalized NMOFs (NMOF-N_3): First, the organic ligand, amino-triphenyl dicarboxylic acid (amino-TPDC), was synthesized according to a reported method.[S1] Afterwards, NMOFs were prepared by heating $ZrCl_4$ with amino-TPDC at 80 °C for 5 days (Figure S1). The resulting NMOFs were collected by centrifugation and washed with DMF, triethylamine/ethanol (1:20, V/V), and ethanol gradually. For the preparation of azide-modified NMOFs (NMOF-N_3), 10 mg of the

dried NMOFs were dispersed in 3 mL of THF, followed by adding 1.0 mL of the tert-butyl nitrite (tBuONO) and 0.9 mL of the azidotrimethylsilane ($TMSN_3$). And then, the reaction mixture was stirred at the room temperature overnight to obtain NMOF-N_3.

Synthesis of DBCO-functionalized nucleic acid (1) (DBCO-DNA): To link DBCO functional groups to nucleic acid (**1**), 80 μL of nucleic acid (**1**) (1 mM) was reacted with 200 μL of DBCO-sulfo-NHS (4 mM) in HEPES buffer (10 mM, pH = 7.4) and the reaction mixture was vigorously shaken overnight. Thereafter, the solution was filtered with MicroSpin G-25 columns (GE-Healthcare) to separate any unreacted DBCO-sulfo-NHS and obtain the pure DBCO-DNA.

Synthesis of nucleic acid (1)-functionalized NMOFs: NMOF-N_3 (10 mg, 2 mL) were added to an aqueous solution of DBCO-modified nucleic acid (**1**) (200 nmol, 1 mL). The mixture was incubated at 40 °C for 72 h, and three portions of a NaCl solution were added to the reaction mixture every two hours within the first 6 hours to reach a final concentration of 0.5 M. Thereafter, the obtained nucleic acid (**1**)-functionalized NMOFs were washed three times with HEPES buffer (10 mM, pH = 7.4) to remove unbound DNA. The UV absorbance of the wash was measured at 260 nm to evaluate the amount of DNA loading on the NMOFs.

Loading of nucleic acid (1)-functionalized NMOFs: The nucleic acid (**1**)-functionalized NMOFs, 5 mg, were incubated with Rhodamine 6G (0.5 mg/mL) or with the anticancer drug, doxorubicin (1.0 mg/mL) overnight in 2 mL of HEPES buffer solution (10 mM, pH = 7.4). Subsequently, the NMOFs were separated and

transferred to a HEPES buffer solution (10 mM, pH = 7.4) that contained NaCl, 20 mM, and NMOFs were hybridized with the nucleic acid (**2**) or (**3**), leading to the locked state of the duplex DNA-functionalized NMOFs loaded with doxorubicin or Rhodamine 6G. After 12 h, the resultant NMOFs were washed several times to remove the excess and nonspecifically bound Rhodamine 6G or doxorubicin.

VEGF-induced unlocking of the NMOFs and the release of the encapsulated loads: Experiments were performed using solutions of the respective Rhodamine 6G or doxorubicin-loaded (**1**)/(**2**)-locked NMOFs or (**1**)/(**3**)-gated NMOFs at a concentration corresponding to 1 mg/mL. Then, the NMOFs solutions, 30 μL, were treated with 10 μL of variable concentrations of VEGF for a fixed time-interval of 30 minutes. Other proteins, e.g. thrombin, hemoglobin, BSA, were used as controls to demonstrate the selective uncaping of the NMOFs by VEGF. After incubation, the respective samples were centrifuged at 10000 rpm for 10 min to precipitate the NMOFs, and the fluorescence of the released loads in the supernatant solution was measured using a Cary Eclipse Fluorescence Spectrophotometer (Varian Inc.).

Cell culture: Malignant breast epithelial cells (MDA-MB-231) were grown in 5% CO_2 RPMI medium 1640 supplemented with 10% FCS, L-glutamine, and antibiotics (Biological Industries). Normal breast cells (MCF-10A) 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 cholera toxin (CT, 0.1 μg/mg), horse serum (5%), insulin (10 μg/mL), hydrocortisone (500 ng/mL), penicillin/streptomycin (1 unit/mL), and epidermal growth factor (20 ng/mL). Cells were plated one day prior

to the experiment on 24-well plates or on microscopic slides glued to perforated 3 cm diameter tissue culture plates for cell viability or microscopic measurement.

Cell uptake of NMOFs: Normal breast cells (MCF-10A) and malignant breast epithelial cells (MDA-MB-231) were planted at a density of 8×10^5 cells in glass-bottomed petri-dish (9.5 cm^2 growth area) with 1 mL medium and incubated with the DOX-loaded NMOFs (6 μg/mL) in the complete growth medium for 6 h. Then the cells were washed with DMEM-HEPES intensively and the nuclei were stained with Hoechst 33342. The fluorescence of DOX was measured by epi-fluorescence microscopy (Nikon TE2000 microscope) equipped with opti-grid. Image analysis was performed using Image J and Volocity programs.

Cell viability experiments: MCF-10A or MDA-MB-231 cells were seeded at a density of 1.8×10^5 cells/well in 24-well plates for assaying cell viability after incubation with different NMOFs. Cells were incubated with or without DOX-loaded NMOFs for 6 hours. Following intensive washing, the cells were further incubated for three days with growth medium and the cell viability was determined after one days, two days and three days with the fluorescent redox probe, Alamar Blue. The fluorescence of Alamar Blue was recorded on a plate-reader (Tecan Safire) (λ_{ex} = 560 nm; λ_{em} = 590 nm).

NMOFs-induced cytotoxicity of 3D-spheroids of cancer cell: For the 3D-spheroid formation and spheroid cells experiments with NMOFs, we have used the IncuCyte Zoom System in the following way. The MDA-MB-231 cells were plated in quadruplicate at 2×10^3 cells per well in a 96 well ULA plate (Corning 7007) and the

cells were further cultured for 3 days to form spheroids. Then, the spheroids were treated with the different types of NMOFs and the cytotoxicity of spheroids was assayed using IncuCyte cytotoxicity probe (IncuCyte® Red Cytotoxicity Reagent, EssenBioscience Cat #4632). Spheroids were imaged at × 10 magnification in an IncuCyte Zoom Live-cell analysis system (Essen Bioscience) at 37 °C with 5% CO_2. Spheroid cell images were recorded every two hours and the resulting red colors, reflecting cell death, were analyzed with the IncuCyte software.

Supplementary References

[S1] He, C.; Lu, K.; Lin, W. *J. Am. Chem. Soc*. **2014**, *136*, 12253-12256.

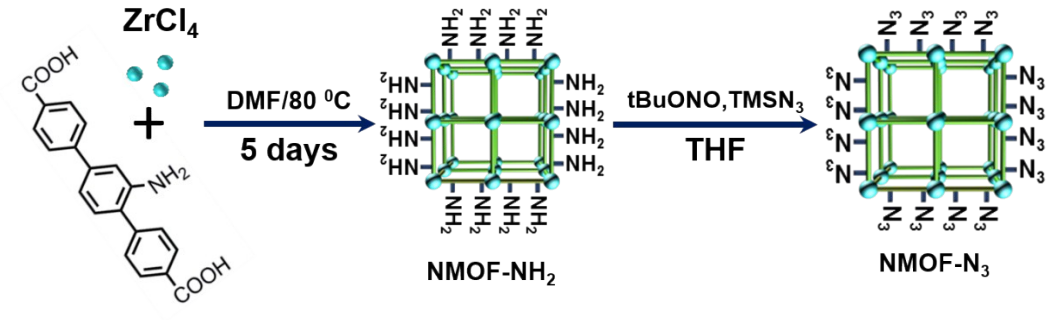

Fig. S1. Synthesis of the NMOFs and their functionalization with azide groups.

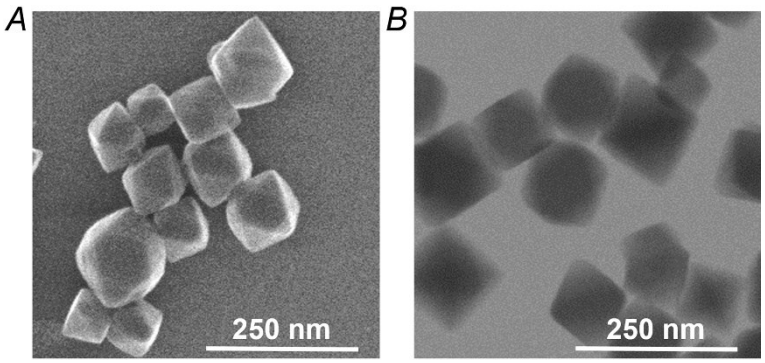

Fig. S2. (A) SEM image of the unmodified NMOFs. (B) TEM image of the unmodified NMOFs.