# Supporting Information

## DNA Tetrahedra Modules as Versatile Optical Sensing Platforms for Multiplexed Analysis of miRNAs, Endonucleases and Aptamer-Ligand Complexes

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## Experimental Section

**Materials.** Magnesium chloride was purchased from Sigma-Aldrich. Tris-borate-EDTA (5×TBE) buffer solution was purchased from Biological Industries. The endonucleases including Nt.BbvCI, HindIII and EcoRI were purchased from New England Biolabs, and NEBuffer 2 was provided by New England Biolabs. The ATP, thrombin and VEGF were purchased from Sigma-Aldrich. GeneRuler 1 kb DNA Ladder was purchased from Thermo Fisher Scientific (USA). DNA/RNA oligonucleotides were synthesized and purified by Integrated DNA Technologies, Inc. "GelRed nucleic acid gel stain" was purchased from New Biotechnology Ltd. Ultrapure water from NANOpure Diamond (Barnstead) source was used in all of the experiments.

The oligonucleic acid sequences used in the study include:

(1): 5'-FAM-CGCCATAGTAGACGTATCACCAGGCAGTTGAGACGAACATTC CTAAGTCTGAAATTTATCACC-BHQ1-3'

(2): 5'-ROX-CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGG TCCAATACCGACGATTACAG-BHQ2-3'

(3): 5'-Cy5-TAGAGACGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGA TACGAGAGCC-BHQ2-3'

(4): 5'-ACGTGTAGCAAGGAAACCCAGCAGACAATGTAGCTGTTTCCTGTAA TCGACTCTAACCCCTATCACGATTAGCATTAAGGGGTGGCTCACTACTATG GCGTCAACATCAGTCTGATAAGCTAGTTGAGGTGATAAAT-3'

(5): 5'-ACGTGTAGCAAGAGAATTCCCTTTGGGAATTCTCTGTAATCGACTCT ACAAGCTTCCTTTGGAAGCTTGGGCTCACTACTATGGCGTCCTCAGCCCTT TGGGCTGAGG AGGTGATAAAT-3'

(6): 5'-ACGTGTAGCAAGGTTCATTCTACCATGAACCTGTAATCGACTCTACT TGATAGTCACCATCAAGGGCTCACTACTATGGCGACCTGGGAATACTCCC CCAGGTGGTGATAAAT-3'

miRNA-221: 5'-AGCUACAUUGUCUGCUGGGUUUC-3'

miRNA-21: 5'-UAGCUUAUCAGACUGAUGUUGA-3'

miRNA-155: 5'-UUAAUGCUAAUCGUGAUAGGGGU-3'

x': 5'-TGG GGGAGTATTGCGGAGGAAGGT-3'

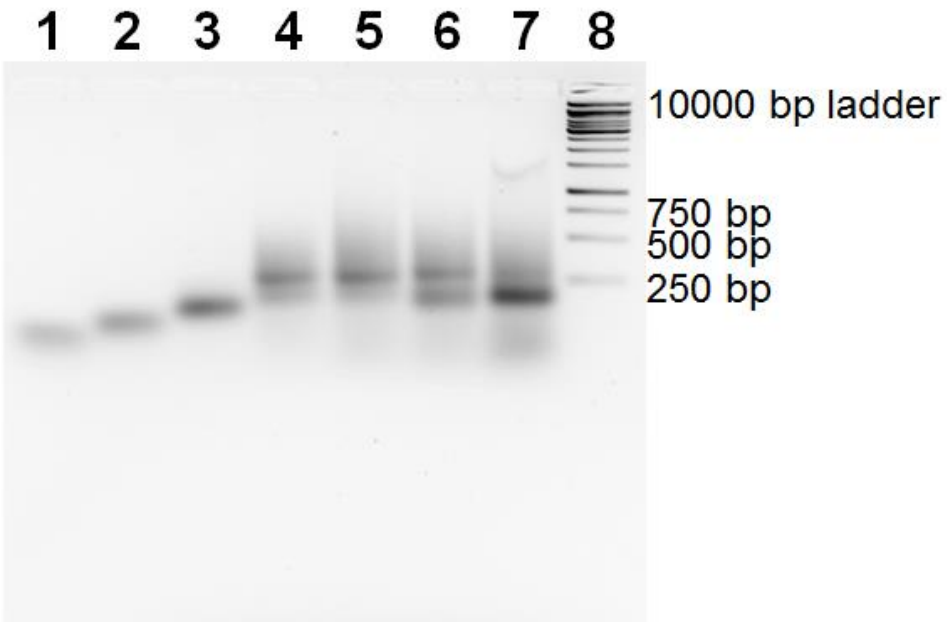
y': 5'-TGTGGGGGTGGACGGGCCGGGTAGAATGAAC-3'

z': 5'-AGTCCGTGGTAGGGCAGGTTGGGGTGACTATCAAG-3'

**Measurements.** The fluorescence spectra of FAM, ROX and Cy5 were collected from 490 to 800 nm with 480 nm excitation, 580 to 800 nm with 570 nm excitation, and

640 to 800 nm with 630 nm excitation, respectively. Fluorescence spectra were carried out on a Cary Eclipse Fluorometer (Varian Inc.).

**Figure S1** shows the agarose (2%) electrophoretic separated bands corresponding to the stepwise assembly of the tetrahedra shown in Scheme 1, and the bands corresponding to the tetrahedron module upon the multiplexed analysis of miRNAs. Lane 1-strand (**1**); Lane 2-duplex strand (**1**) + (**2**); Lane 3-Y-shaped structure consisting of strands (**1**) + (**2**) + (**3**); Lane 4-intergrated tetrahedra module composed of (**1**) + (**2**) + (**3**) + (**4**); Lane 5-Intergrated tetrahedra modified with miRNA-221; Lane 6-Intergrated tetrahedra module carrying miRNA-155 + miRNA-221; Lane 7-Intergrated tetrahedra module treated with miRNA-21 + miRNA-155 + miRNA-221; Lane 8-DNA ladder.




The results shown in lane 1 to lane 4 show the stepwise assembly of the tetrahedra module composed of the strands (**1**) + (**2**) + (**3**) + (**4**). The tetrahedra module reacted with miRNA-221 in lane 5 reveals intact tetrahedra module of slightly elevated molecular weight, consistent with the carrying of the hybridized miRNA-221. The band shown in lane 6 shows a band of slightly higher molecular weight, demonstrating an intact tetrahedra structure carrying the two miRNAs (miRNA-221 and miRNA-155). Lane 7 reveals that the tetrahedron module treated with the three miRNAs (miRNA-21, miRNA-155 and miRNA-221) yield a band of lower molecular weight presumably of a mixture consisting of the Y-shaped core (**1**) + (**2**) + (**3**), and the frame (**4**) linked to the three miRNAs. The separation of the tetrahedra might originate from the strain affected on the distorted tetrahedra upon binding the three miRNAs or harsh conditions of the electrophoretic separation.

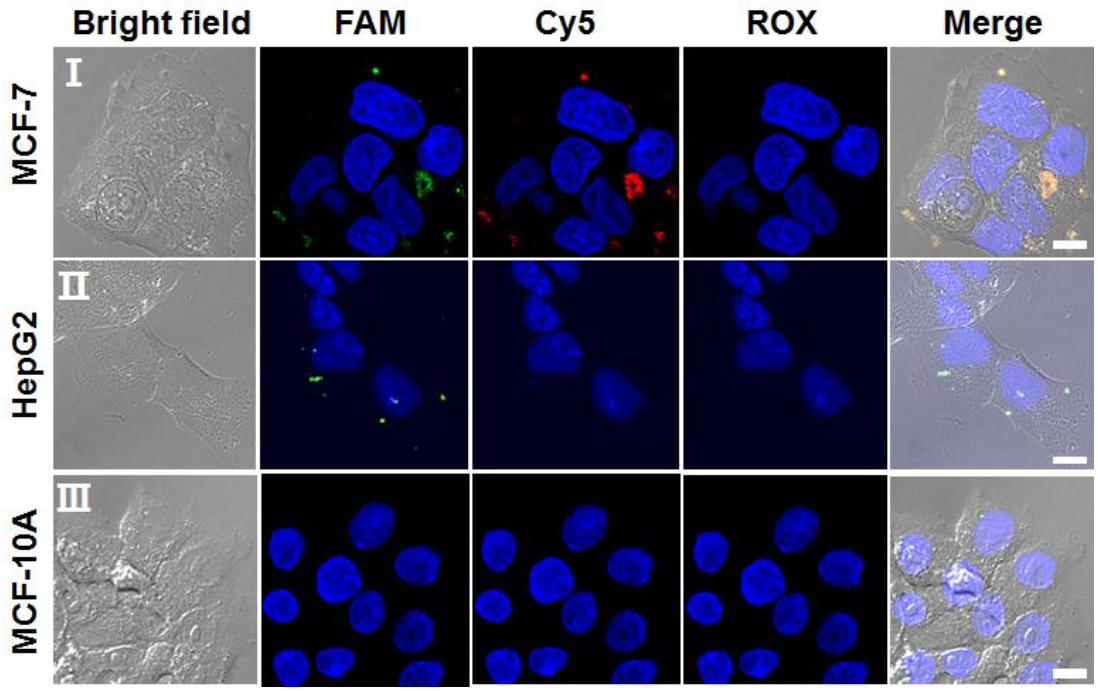



**Figure S2.** Large-area bright-field microscopy and confocal fluorescence microscopy imaging domains corresponding to: Entry I-the MCF-7; Entry II-the HepG2; Entry III-the MCF-10A cells treated with the FAM/Cy5/ROX-functionalized tetrahedra. The fluorescence of the three fluorescent probes was imaged through the channels: FAM emission, $\lambda_{ex}$ = 488 nm; Cy5 emission, $\lambda_{ex}$ = 640 nm; ROX emission, $\lambda_{ex}$ = 561 nm. Scale bar: 10 μm.

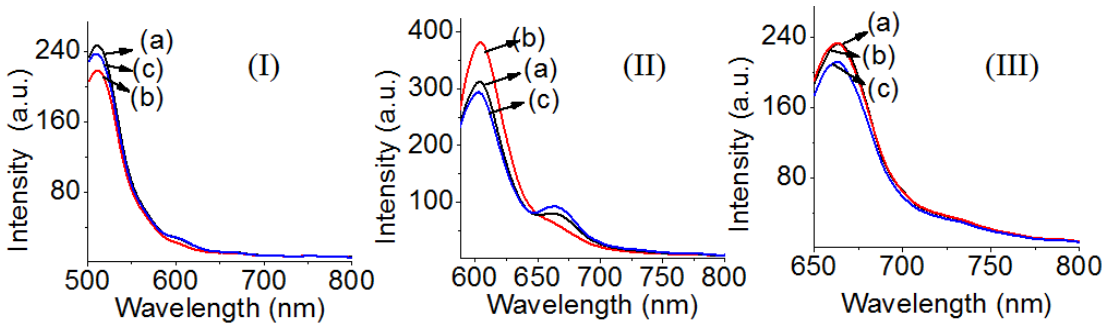



**Figure S3.** Panel I-fluorescence spectra of FAM: (a) in the absence of added ATP; (b) upon the addition of VEGF; (c) upon addition of thrombin. Panel II-fluorescence spectra of ROX: (a) in the absence of added VEGF; (b) upon the addition of ATP; (c) upon addition of thrombin. Panel III-fluorescence spectra of Cy5: (a) in the absence of added thrombin; (b) upon the addition of ATP; (c) upon addition of VEGF.