Supporting Information for: "DNA-based delivery vehicles: pH-controlled disassembly and cargo release" by Jung-Won Keum and Harry Bermudez

DNA sequences

Strand	Sequence
1-0	GGGTTAGGGTTAGGGTTAGGGAGGGTTAGGGTTAGGGTTAGGGAGGGTTAGGGTTAGGGTTAGGG
1-1	GGGTTAGtGTTAGGGTTAGGGAGGGTTAGGGTTAtGGTTAGGGAGGGTTAGGGTTAGGGTTAGtG
1-2	GGGTTAGtGTTAtGGTTAGGGAGGGTTAGGGTTAtGGTTAGtGAGGGTTAGtGTTAGGGTTAGtG
1-3	GGGTTAGtGTTAtGGTTAGtGAGGGTTAGtGTTAtGGTTAGtGAGGGTTAGtGTTAtGGTTAGtG
1-4	tGTTAtGGTTAGGGAGGGTTAGGGTTAtGGTTAGtGAGGGTTAGtGTTAGGGTTAGtGAGGGTTAG
2	CCCTAACCCTAACCCTAACCCATGACGAAGACCTTCTCGGCCGAGAGCGCATGGTGGTTCGGCCA
3	CGGCCGAGAAGGTCTTCGTCAACCCTAACCCTAACCCTAACCCACACCCTCCGTCCCGGGCGCTC
4	CCCTAACCCTAACCCTAACCCATGGCCGAACCACCATGCGCTCAGAGCGCCCGGGACGGAGGGTG
5	TTACGACGAGACACATGGGAACCGAACAATGTCGGAACGGCAGGCCAATGCTCACGGCGGAGA
6	GGAACGTCCGAATGATATAAACTCCGCCGTGAGCATTGGCCATGAAACTCCCAGCGAGCAGCA
7	GCCGTTCCGACATTGTTCGGAGTAGAACTTAACAGGGAAGCAGCTGCTCGCTGGGAGTTTCAA
8	TCCCATGTGTCTCGTCGTAAATTATATCATTCGGACGTTCCAGCTTCCCTGTTAAGTTCTACA
9	TCTCCGCCGTGAGCATTGGCCTGCCGTTCCGACATTGTTCGGTTCCCATGTGTCTCGTCGTAA

Table S1: All sequences follow the usual convention that left-to-right corresponds to a 5'-to-3' orientation. Unpaired A's and T's are indicated by underlines while mismatches are indicated by lowercase font. Strands 1–4 assemble to form pyramids with i-motifs, strands 5–8 assemble to form pyramids without i-motifs, and strand 9 is complementary to strand 5.

Materials and assembly of DNA pyramids

All oligonucleotides were purchased from Integrated DNA Technology. Stoichiometric quantities of the component DNA strands were mixed in TM buffer (10 mM Tris, 5 mM $MgCl_2$ (pH 8.8 or pH 5.0)) to a final total concentration of 0.4 μM. DNA solutions were heated at 95°C for 5 minutes, followed by cooling rate of -1°C per 30 sec and finally to 4°C. The pH of the DNA mixture was adjusted using 0.5 M HCl or 0.1 M NaOH. Assembled pyramids at different pH were analyzed with 5% native polyacrylamide gel electrophoresis at 4°C in 1$\times$ TBE buffer.

pH cycling experiments

For switching from alkaline to acidic conditions, 0.5 M HCl was added to reach pH 5. For switching from acidic to alkaline conditions, 0.1 M NaOH was added to reach pH 8.5, followed by heating at 60°C for approximately 10 min.

Circular dichroism (CD)

CD spectra of pH responsive DNA pyramid (0.2 μM) were measured on a JASCO J-720 spectropolarimeter with quartz cuvettes at 20°C. The spectra were recorded from 220 to 320 nm as an average of 3 scans at a rate of 20 nm min^{-1}. CD spectra of hybridization buffer were subtracted to eliminate background effects.

Preparation of NTA-modified DNA

Thiol-modified strand 1-2 (200 μM) in HEPES buffer (20 mM HEPES, 150 mM NaCl, pH 7.5) was reduced by 20 mM TCEP solution (Sigma). 10 mg of maleimido-C3-NTA (Dojindo, Japan) was added to DNA solution and incubated at room temperature for 20 hr.

Preparation of EGFP with His tag

The plasmid pDHM EGFP that encodes EGFP was kindly provided by Dr. Jin-Ho Ahn. EGFP gene is cloned between NdeI and KpnI sites and the plasmid is kanamycin resistant. The plasmid was amplified in DH5α and expressed in BLR De3 cells (Novagen) through IPTG induction. His-EGFP was purified from the crude extract using Ni-NTA agarose (Qiagen). To remove imidazole in eluted EGFP and achieve buffer exchange to PBS, a size exclusion spin column (Biorad, Micro Bio Spin 6) was used.

Formation of EGFP-incorporated DNA pyramids

DNA pyramids were initially assembled in TM buffer (20 mM Tris, 30 mM $MgCl_2$, pH 8.8) using slow cooling from 95°C to 4°C with a rate of -1°C per 30 sec. 2 μL of 10 mM $NiCl_2$ solution was added to 800 μL of 0.8 μM DNA solution and incubated for 10 min. Next, 10 μL of 10 μM EGFP solution was added to DNA solution and incubated at 4°C for 3 hours with shaking. To remove excess unbound EGFP, 10 μL of Ni-NTA agarose (Qiagen) was included in the mixture and incubated for 1 hr. Ni-NTA bead was removed by passing through polypropylene column (1 mL, Qiagen) and flow-through was analyzed using a fluorescence spectrometer (Perkin Elmer).

Fluorescence measurements

0.4 μM of assembled pyramid with either modified with TAMRA and IabRQ pair or EGFP and Cy3 pair were analyzed using a quartz cell (Starna) on a fluorescence spectrometer (Perkin Elmer). For TAMRA and IabRQ quenching experiments, samples were excited at 555 nm with both excitation and emission band width of 5 nm, and fluorescence emission was collected with scan rate of 10 nm sec^{-1}. For EGFP and Cy3 FRET experiments, samples were excited at 480 nm with both excitation and emission band width of 10 nm, and fluorescence emission was collected with scan rate of 100 nm sec^{-1}.

PAGE verification of DNA pyramid assembly

Figure S1: Native gel electrophoresis of i-motif DNA pyramids. Lane 1: strand 2; Lane 2: strand 2 and strand 3; Lane 3: strands 2–4; Lane 4: strands 1–4 (pyramids).

Effect of pH on DNA hybridization

Figure S2: Assembly of DNA pyramid lacking i-motifs (strands 5–8) and hybridization of linear dsDNA (strands 5 and 9) is unaffected by different pH. Lane 1: pyramids under alkaline conditions; Lane 2: pyramids under acidic conditions; Lane 3: linear dsDNA under alkaline conditions; Lane 4: linear dsDNA under acidic conditions.

Fluorescence quenching experiments

Strand 1 and strand 3 were labeled with Iowa Black RQ (IabRQ) and TAMRA, respectively. Neither dye modification interfered with the assembly of the DNA pyramids as shown by PAGE (Figure S3a). We observed small changes in TAMRA fluorescence depending on pH, and moreover, TAMRA fluorescence depends on whether it is attached to single-stranded DNA or double-stranded DNA (Massey et al., 2006). Since these properties complicate the interpretation of FRET data, only identical secondary structures were compared at different pH values. Under alkaline conditions, presence of the IabRQ quencher decreases TAMRA fluorescence by 16% ($n = 2$), verifying the successful assembly of i-motif pyramids under alkaline conditions (Figure S3b). Given the Förster distance R_o for the TAMRA-IabRQ pair is 4.3 nm (Massey et al., 2006) and the energy transfer efficiency $E = R_o^6/(R_o^6 + R^6)$, the fluorophore-quencher separation R was calculated to be 5.6 nm. Since the fluorophore and quencher are attached via flexible linkers to the corners of a pyramid having 6.8 nm-long edges, this value of R agrees reasonably well with our design.[1] When dual-labeled i-motif pyramids are exposed to acidic conditions, our design is intended for IabRQ-labeled strand 1 to dissociate and thereby allow TAMRA fluorescence recovery. To demonstrate the efficiency of strand 1 dissociation under acidic conditions, the fluorescence of dual-labeled pyramids was compared to the "trimer" structure corresponding to complete dissociation of strand 1. Although the fluorescence of TAMRA itself was slightly affected by pH, the two structures under acidic conditions show very similar fluorescence emission (Figure S3c), suggesting that strand 1 achieved efficient dissociation from the pyramids.

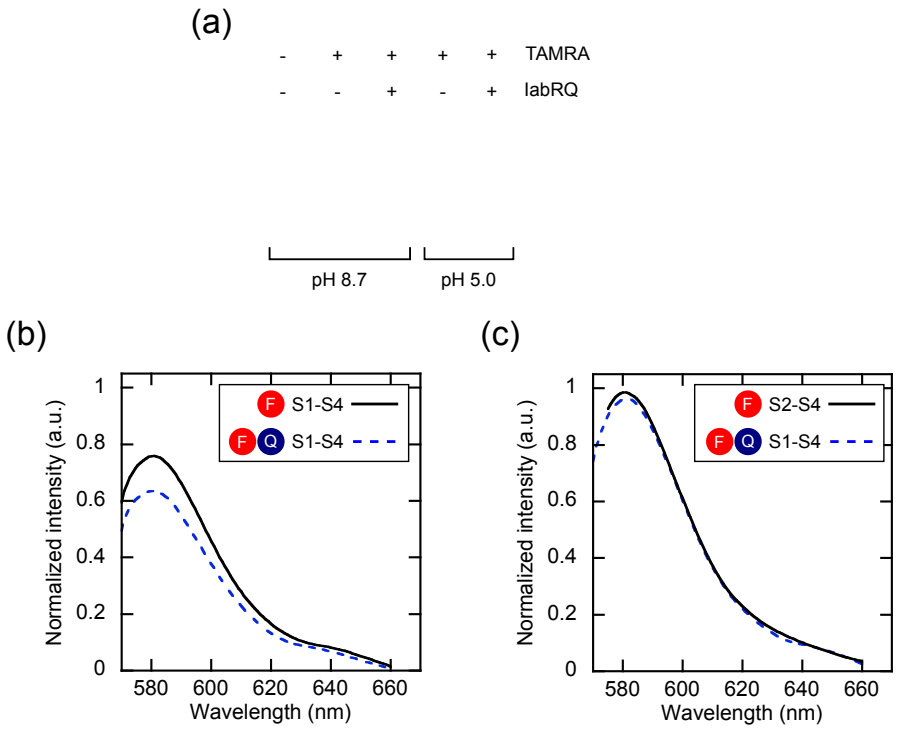

Figure S3: (a) Native gel analysis of TAMRA- and IabRQ-labeled i-motif pyramids at different pH reveals no inhibition of pyramid assembly under alkaline conditions. Under acidic conditions, the i-motifs cause disassembly, as expected. Fluorescence quenching between TAMRA and IabRQ to verify conformational changes in i-motif DNA pyramids at different pH ("F"= TAMRA and "Q"= IabRQ). Normalized fluorescence data of labeled i-motif pyramids under (b) alkaline conditions and (c) acidic conditions.

[1] According to the supplier, the TAMRA azide linker is about 3 nm in length, and the IabRQ linker is about 2 nm in length.

Control experiments for EGFP-labeled pyramids

Following the incubation of DNA with EGFP, any unbound protein was removed by passing the mixture through Ni^{2+}-NTA agarose beads. When the NTA-modified strand 1 is incubated with EGFP, the resulting solution shows a strong emission peak around 510 nm, indicating the presence of a DNA-EGFP interaction (Figure S4). In comparison, mixing unmodified DNA with EGFP showed only a minimal EGFP emission peak, indicating no interaction between DNA and EGFP and the effective removal of free EGFP by Ni^{2+}-NTA agarose beads. Similarly, mixing the NTA-modified DNA with EGFP in the absence of Ni^{2+} also failed to show significant fluorescence. As a further test of specificity, pyramids bearing Cy3 but lacking NTA were incubated with free EGFP and showed no FRET signal (Figure S5), further confirming that the intended conjugation was successful.

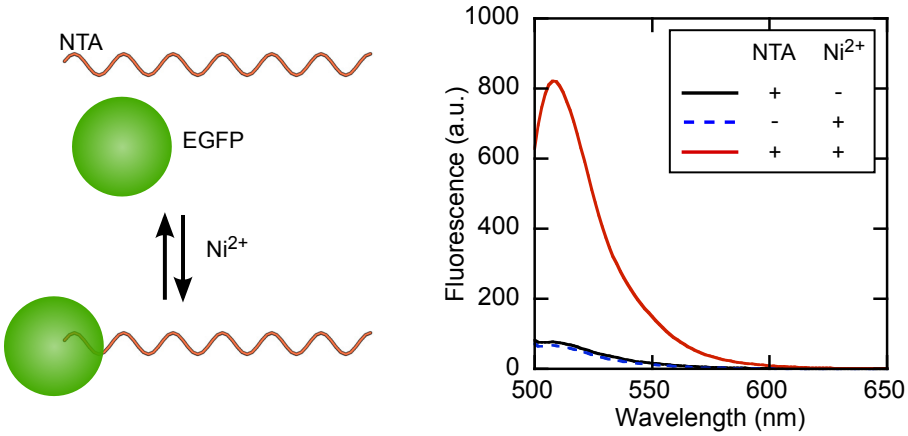

Figure S4: NTA modification to incorporate EGFP into i-motif pyramids. Interactions between DNA and hexa-histidine tagged EGFP in the presence or absence of NTA-modification and Ni^{2+} ions.

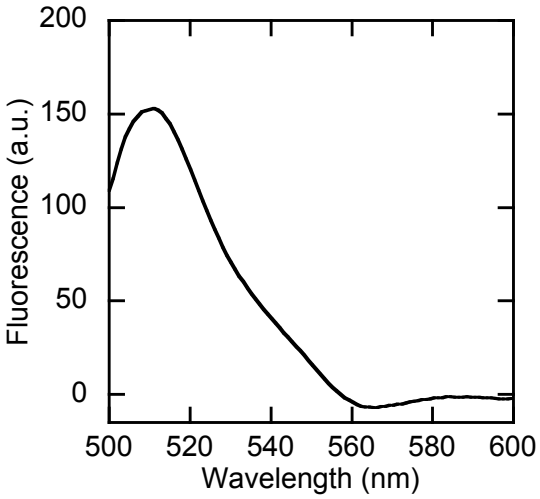

Figure S5: Incubation of Cy3-labeled pyramids (via strand 3) and free EGFP, after subtraction of background signal from Cy3-labeled pyramid. Under these conditions, there is minimal emission from Cy3 and therefore no FRET, as indicated by the signal at 565 nm.

EGFP fluorescence under different pH conditions

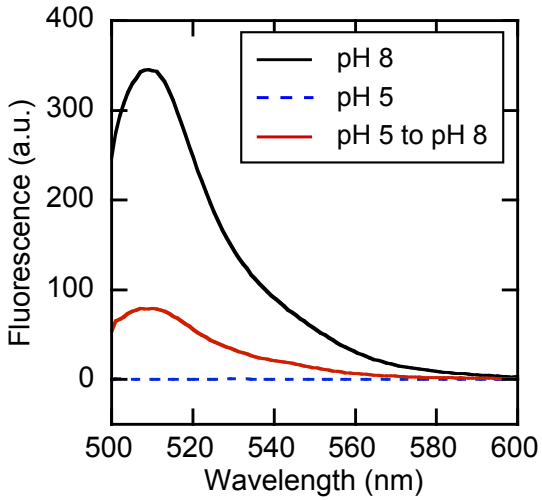

Figure S6: Fluorescence of EGFP in hybridization buffer at different pH conditions. Fluorescence under acidic conditions was partially recovered when conditions are adjusted back to pH 8.

Triggered EGFP release via competitive binders

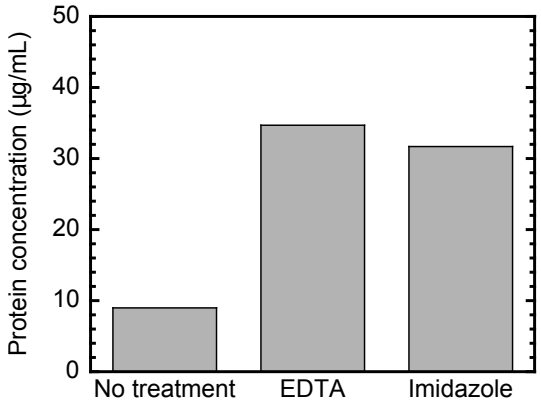

Figure S7: Released EGFP levels after treatment with either EDTA or imidazole were quantified using a Bradford assay.