**Multifunctional nano-architectures from DNA-based ABC Monomers**

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**Synthesis of Y-DNA donor and X-DNA acceptor**

Y-DNA donor and X-DNA acceptor were fabricated according to previously published methods from our group¹⁻³. In a typical experiment, the Y-DNA donor was synthesized by mixing the same molar amount of corresponding oligonucleotide strands. The nomenclature is as follows: Y₀₁, Y₀₂ and Y₀₃ are the three corresponding single oligonucleotide chains that form a Y-DNA. To form fluorescent tagged Y-DNA, commercially synthesized fluorescent tagged oligonucleotide strands were used. Similarly, X₀₁, X₀₂, X₀₃ and X₀₄ are the four corresponding single oligonucleotide chains that form an X-DNA. All strands including biotinylated and amine modified strands were purchased from IDT (Integrated DNA Technologies, Coralville, Iowa).
Conjugation of PEGA onto Y-DNA

Acrylate-(polyethylene glycol)-succinimidyl carboxy methyl ester is a set of compounds having polyethylene glycol (PEG) spacers with monoacrylate (PEGA) and amine-reactive N-hydroxysuccinimide (NHS) -ester groups at opposite ends (Fig. S1). The NHS-ester is spontaneously reactive with primary amines (–NH₂) of Y-DNA, providing an efficient route for conjugation. To tether the PEGA to Y-DNA, 0.5 µM PEGA-NHS (3,400 Da) was added into the solution containing 0.2 µM 5'-amine-modified Y-DNA (NH₂-Y-DNA). The reaction was carried out overnight at room temperature. PEGA-Y-DNA was separated from non-reacted NH₂-Y-DNA and PEGA by an HPLC XBridge C18 column equipped with a photo-diode array detector (Waters). The HPLC chromatogram was obtained (Fig. S2a) from a gradient elution from 0–50% acetonitrile in 0.1 M triethylammonium acetate (TEAA, pH 7.0) as the mobile phase within 40 min at a flow rate of 0.5 mL/min with UV detection (260 nm). Multiple peaks are observed in fig. S2a because the arms of Y-DNA are double helixes, and they can breathe (partially zip and unzip the structure) at the room temperature. However, the

Figure S1. Synthesis of PEGA modified Y-DNA.
resulting products show a single major band in gel electrophoresis in Fig. S2b. Lane 1 indicates NH$_2$-Y-DNA (from HPLC fraction B) and lane 2 indicates PEGA-Y-DNA (from HPLC fractions C1-C2). Compared to NH$_2$-Y-DNA, the gel electrophoretic mobility of PEGA-Y-DNA was retarded, as expected, because of the increase of molecular weight.

![Figure S2. a, HPLC chromatogram of PEGA and Y-DNA conjugation reaction. The separation of PEGA-NHS (A), NH$_2$-Y-DNA (B), and PEGA-Y-DNA (C1, C2) were achieved by a gradient elution. b, Gel electrophoresis (3% agarose gel, EtBr staining) of HPLC fractions. Lanes 1 and 2 indicate NH$_2$-Y-DNA and PEGA-Y-DNA, respectively.](image)

FTIR spectrum of PEGA-Y-DNA (Fig. S3) revealed several transmission bands from an amide bond, PEG, and DNA. Two characteristic bands of C=O and N-H groups were observed at 1672 and 1620 cm$^{-1}$ for amide bond. The transmission bands at higher wavenumbers were assigned to the amide I band and the bands at lower
wavenumbers to the amide II band, which is typical of primary amides. Another transmission band at 2881 cm\(^{-1}\) was assigned to the CH\(_2\) group of PEG. The transmission bands at 1080 and 1252 cm\(^{-1}\) were assigned to the PO\(_2\) group of DNA. The appearance of a new amide bond confirmed the formation of PEGA-Y-DNA.

**Figure S3.** FTIR spectra (KBr) of PEGA-Y-DNA in the region of 3000 to 1000 cm\(^{-1}\). [Transmittance bands at 1080 and 1252 cm\(^{-1}\) in both spectra belong to PO\(_2\) of DNA; 1620 cm\(^{-1}\) belongs to N-H of amide bond (amide II) and 1672 cm\(^{-1}\) belongs to C=O of amide bond (amide I). 2881 cm\(^{-1}\) belongs to CH\(_2\) of PEG].

**Synthesize ABC monomer with fluorescence dyes and investigate the selectivity**

To show the selectivity of our approach, seven different ABC monomers were synthesized and characterized via gel electrophoresis (3% agarose gel at 90 volts at 25°C in Tris-acetate-EDTA (TAE) buffer (40 nM Tris, 20 nM acetic acid and 1 mM...
EDTA, pH 8.0, Bio-Rad, Hercules, CA)). First, 1.5 µM X-DNA and three different types of 1.5 µM Y-DNA (one green (1G), two reds (2R), and one green and one red (1G1R)) were incubated (in Fig. S4a). Second, specific Y-DNA was connected to X-DNA by adding 1.5 µM specific bridge DNA which was complementary to the sticky end of both X- and Y-DNA. Without bridge DNA, X- and Y-DNA were not linked (Lane 1 in Fig. S4b). For example, only 1G Y-DNA were connected to X-DNA by adding w bridge DNA. Both 1G and 2R Y-DNA were liked to one X-DNA by adding w and n bridge DNA. The ABC monomer, which consisted of three Y-DNA by adding all three bridge DNA, was selected for further experiments (Lane 8 in Fig. S4b).
Figure S4. a, Schematic drawing of synthesizing ABC monomers by controlling multimoieties onto a single anisotropic X-DNA. We first prepared three different types of Y-DNA (designated as \( W, N, \) and \( E \)) that carried three different configurations of fluorescence dyes: one green (1G), two reds (2R), and one green and one red (1G1R). In addition, three single stranded DNAs (designated as \( w, n, \) and \( e \) in lower case letters) were uniquely designed in such a way that half of the sequence was complementary to the corresponding Y-DNA’s sticky end, and the other half was complementary to one of the X-DNA’s end-sequence (\( W', N', \) and \( E' \)). Thus, these three single stranded DNA served as “bridge DNA” that linked X and Y together in an anisotropic and specific manner: \( w, n, \) and \( e \) only linked \( W'-w-W', N'-n-N', \) and \( E'-e-E' \), respectively. By simply adding a specific bridge DNA, different fluorescence dyes were donated onto the corresponding branches of a single X-DNA with nanometer precision. b, Gel electrophoresis migration pattern of ABC monomers after Ethidium Bromide (EtBr) staining. Lane 1 corresponds to X-DNA (top) and non-reacted Y-DNA (bottom). Note that X-DNA itself is not shown in gel electrophoresis images before EB staining. Lanes 2, 3, and 4 correspond to X-DNA connected with one Y-DNA (top). Lanes 5, 6, and 7 correspond to X-DNA connected with two Y-DNA (top). Lane 8 indicates that all three Y-DNA are connected onto X-DNA with all three bridge DNA.

Conjugation of quantum dots (QDs) with Y-DNA

QDs were from Invitrogen (Carlsbad, CA). The QDs were commercially coated with streptavidin. The emission wavelengths of the green QD (Qdot® 525 streptavidin conjugate) and the red QD (Qdot® 655streptavidin conjugate) were 525nm
Figure S4. a, Schematic drawing of synthesizing ABC monomers by controlling multi-moieties onto a single anisotropic X-DNA. We first prepared three different types of Y-DNA (designated as \( W \), \( N \), and \( E \)) that carried three different configurations of fluorescence dyes: one green (1G), two reds (2R), and one green and one red (1G1R). In addition, three single stranded DNAs (designated as \( w \), \( n \), and \( e \) in lower case letters) were uniquely designed in such a way that half of the sequence was complementary to the corresponding Y-DNA's sticky end, and the other half was complementary to one of the X-DNA's end-sequence (\( W' \), \( N' \), and \( E' \)). Thus, these three single stranded DNAs served as "bridge DNA" that linked X and Y together in an anisotropic and specific manner: \( w \), \( n \), and \( e \) only linked \( W'-w-W \), \( N'-n-N \), and \( E'-e-E \), respectively. By simply adding a specific bridge DNA, different fluorescence dyes were donated onto the corresponding branches of a single X-DNA with nanometer precision.

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**Figure S5.** Schematic drawing of the structure and composition of QD used. The QD is made of CdSe, which is then coated with a ZnS shell. The core-shell is further protected with a polymer shell that stabilizes QD in aqueous solution. Streptavidin are then conjugated to the outer polymer shell.

**Synthesize ABC monomers with nanoparticles**

To synthesize ABC monomers with three quantum dots, the south branch of the X-DNA was first anchored onto a solid bead 1 (Fig. S6a). Both west and east end-
sequences of X-DNA were then connected with Y-DNA donor tethering green quantum dots. The end-sequence at the north of an X-DNA was connected with red QD Y-DNA. Then, the ABC monomers attached to the beads 3 were released by a restriction enzyme Dde I digestion and the isolated ABC monomers 4 were collected. This solid phase synthesis was modified according to previously reported methods from our group.\(^6\)

**Figure S6.** a, Schematic drawing of ABC monomer synthesis with nanoparticles. b, Microsphere beads labeled with 3 are observed by digital camera. c, After isolation of 4 by restriction enzyme, entire solution emits fluorescence indicating that ABC monomers are separated successfully from the beads.

**Scanning Transmission Electron Microscopy (STEM) Imaging**

Samples were prepared by placing a 10 µl drop of the ABC monomer solution onto a copper grid coated with an ultra-thin carbon film and allowed to evaporate. Once the solution was completely evaporated, the sample was exposed to UV light for approximately 40 minutes to prevent contamination build-up during microscopy.
STEM images were obtained on a 200 keV Tecnai F20 microscope with 1.6 Å resolution in annular dark-field STEM mode.

**Figure S7.** a, The distance between two QDs is calculated by considering a rigid DNA model with 0.34 nm per base pair. b, The yield of ABC monomers anchoring three QDs are evaluated by STEM images.

**Figure S8.** a, Fluorescence spectrum of individual green QDs and red QDs as the reference. b, spectrum of synthesized 1G1R ABC monomers. Note that the ratio of 1G1R is well matched with the reference.
**Figure S9.** Dynamic light scattering data of the ABC monomers and polymeric spheres. 

**a,** The increase of size of the polymeric spheres after photo-polymerization. **b,** Changes of polymeric sphere sizes with different concentrations of ABC monomers.

**Loading drugs (ODN and siRNA) to polymeric spheres**

To load drug (ODN) in our spherical polymers, the ODNs were hybridized to the sticky end of ABC monomers first. Then, the spherical polymers were able to carry the hybridized ODN in a cluster format by photo-polymerization (Fig. S10).
Figure S9. Dynamic light scattering data of the ABC monomers and polymeric spheres. 

a, The increase of size of the polymeric spheres after photo-polymerization.

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Figure S10. Schematic drawing of nucleic-acid-based drug loading into polymeric spheres with ODN 1 or siRNA 2. Both ODN and siRNA can be loaded in the sphere by hybridization.

Figure S11. Fluorescence microscopic images of HeLa cell treated with polymeric spheres (blue: nuclei, green: actin), a, at 4°C overnight, and b, at 37°C for 10 min. Scale bar is 10 µm.
Investigation of endocytosis mechanism of the polymeric spheres uptake

To investigate the cellular endocytosis mechanism of the polymeric spheres, HeLa cells (5 x 10⁴) were cultured in each well on Lab-Tek chamber slide (8 wells, Permanox slide, Nunc) overnight. Cells were pre-incubated with endocytosis inhibitors for 30 min with 10 µg/mL of chlorpromazine hydrochloride (sigma) and 5 µg/mL of filipin complex (sigma), and for 1 hr with 0.5 µg/mL of Cytochalasin B and 0.1% of DMSO as a positive control. Cells were then cultured with 20 µL of polymeric spheres (2.9 pM) and each inhibitor for 3 hrs. The number of cells uptaking the spheres was counted from fluorescent microscope images after staining with the same method described in materials and method in the main manuscript.

The cellular endocytosis mechanism of polymeric spheres was studied by inhibition experiments. Cells were incubated with chlorpromazine⁷,⁸ to inhibit the clathrin-mediated endocytosis pathway and with filipin complex⁷,⁸ to inhibit caveolae-mediated endocytosis. Cytochalasin B was used to disrupt actin-mediated endocytosis⁹,¹⁰. As seen in Fig. S12, chlorpromazine and filipin did not reduce the uptake of spheres, while cytochalasin B significantly reduced cellular uptake of the spheres. According to the results, we believe that actin-dependent endocytosis such as phagocytosis and macropinocytosis is the main mechanism for the cellular uptake.
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Figure S12. Cellular uptake of polymeric spheres in the presence of various endocytosis-specific inhibitors.
Supplement Table 1. Oligonucleotide sequences of the DNA building blocks for ABC monomers with fluorescent dyes.

| Strand | Sequence |
|--------|----------|
| X DNA | X01 5’-CGACCGATGAATAGCGGTCAGATCCGTACCTACTCGCTCA-3’ |
|    | X02(W’) 5’-CGAGACCATAAGCTACAGGACGATCTATTCTAGGTCGTGGTGGTTATAT-3’ |
|    | X03(N’) 5’-CGAGTCGTTTGGCATTGCTACTATGTGCTCTCGCTCTACCTGT-3’ |
|    | X04(E’) 5’-/Phos/-CGAGTCGATCCAGATCGTTGACATGATCCGGCTCTCTCATGACCC-AG-3’ |
| Bridge DNA (w) | 5’-CATGTCAGTAGATTATAACCACCA-3’ |
| Bridge DNA (n) | 5’-AGATGCAATAGGAATCAGGTAGAGACGAG-3’ |
| Bridge DNA (e) | 5’-ATAATACGTGCTTTGGTTCACAGC-3’ |
| Y DNA | Y01(W) 5’-AATCAGCTACATGTTGATCGCATACAGGCTCGTAAAG-3’ |
|    | Y02 ● 5’-Alexa Fluor 488 ● -CTTACGGCAATGACCAGATCCGTCTA-3’ |
|    | Y03 5’-AGGCTGATTCGTTTCATCGCATCCA-3’ |
| Y DNA | Y01(N) 5’-TACTATTGCAATCCTGATCCCGATACATTGCGTAAAG-3’ |
|    | Y02 ● 5’-BODIPY630/650 ● -CTTACGCGAATGACCGATCCGTCTA-3’ |
|    | Y03 ● 5’-BODIPY630/650 ● -AGGCTGATTCGTTTCATCCGTCCA-3’ |
| Y DNA | Y01(E) 5’-ACGCAGTTATTACGATCCAGATCCGCTCGTAAAG-3’ |
|    | Y02 ● 5’-Alexa Fluor 488 ● -CTTACGCGAATGACCGATCCGTCTA-3’ |
|    | Y03 ● 5’-BODIPY630/650 ● -AGGCTGATTCGTTTCATCCGTCCA-3’ |
| SP DNA | SP01 5’-Biotin-C6-CCGGATAAGGCGCAAGCGCTGCTGATAATTACAGGTGTTGTCCTG-3’ |
|    | SP02 5’-/Phos/-GTGAGGTACCGTAAAGCTTCTGGCTCCTCAAAGGTGTGCTGGCCTTATCGC-3’ |

Note that /phos/ represents the phosphorylation on the 5’ end of the oligonucleotide. The labeled fluorescent dye is represented by a dot with the same color. The same colored sequences represent complementary pairs.
Supplement Table 2. Oligonucleotide sequences of the DNA building blocks for ABC monomers with nanoparticles.

| Strand | Sequence |
|--------|----------|
| X DNA  | X₀₁ 5'-Phos/-TGAGCACCAGATGAAATAGCGGTCAGATCCGCCTACTACTCG-3' |
|        | X₀₂ 5'-Phos/-ATCCCAGTAGGTACGGGATCTCCTCGTAATTGCGAAGCAGCTCG-3' |
|        | X₀₃ 5'-Phos/-GCAACGAGTCTTCGCAATACGGCTTACTGATACGTATGCTCG-3' |
|        | X₀₄ 5'-Phos/-GAGTCGAGACCATACGGCTACGACCGCTTTCTCATCGTGTC-3' |
| Y DNA (QD) | Y₀₁ 5'-GGCABCATGCACGGCGATGAGGTAACATCGCGTACCCGATGCAC-3' |
| Y DNA (AuNP) | Y₀₂ 5'-GTGTTGTACCGCGAATGTCGTCACAGCAGCAGCTGCGA-3' |
|        | Y₀₃ 5'-Phos/-GGATTCGACAGGCTGATTCGCTGCTACCTCATGCGGATCAGTCGC-3' |
| SP DNA | SP₀₁ 5'-Biotin- C₆-CCGGATAAGGCCAGCGGTCGGCTGAATTCAGGGTTCGTGGCAGGCCAGCACACTTGGAGACCGAGCAGCTC-3' |
|        | SP₀₂ 5'-Phos/-TCAGTTAGGAGTCCCGTAAGCTTCGTCAGCTCAGGCTGGCCCTGCACGACACTGAGCAGC-3' |

Note that /phos/ represents the phosphorylation on the 5' end of the oligonucleotide. The labeled fluorescent dye is represented by a dot with the same color. The same colored sequences represent complementary pairs.
### Supplement Table 3. Oligonucleotide sequences of pathogen DNA and DNA building blocks for target-driven polymerization (Ban = Anthrax, Ebo = Ebola, Sars = SARS)

| Strand                        | Sequence                                                                 |
|-------------------------------|--------------------------------------------------------------------------|
| **Target (Ban) DNA**          | 5’-GGATTATTGTTAAATATTGATAAGGAT-3’                                       |
| **Target (Ebo) DNA**          | 5’-CATGTACGTGATTATAAACCCACA-3’                                          |
| **Target (Sars) DNA**         | 5’-ATAATACCTGCCTTTGTTACAGC-3’                                           |
| **Unrelated DNA**             | 5’-AGATGCAATAGTAATCAGGTAGAGACG-3’                                       |
| **Y01(Ban)**                  | 5’-TTAACAATAATCTCCTTTACGGCGAATGTCGTCACACGCACACGCGATCCAGCTGC-3’           |
| **Y02(Biotin)**               | 5’-Biotin-C<sub>6</sub>-GTGTGCTTTACGCGAATGTCGTCACACGCACACGCGATCCAGCTGC-3’|
| **Y03(Ban)**                  | 5’-/Phos/-GGATTCGACAGGGCTGTATCAGGCTGATTCGGTGCTTACCTCATGCGGATCCAGCTGACG-3’|
| **Y01(Ebo)**                  | 5’-GCCACTGGATCCGATGAGGTAATGTCGTCACACGCACACGCGATCCAGCTGC-3’              |
| **Y02(Biotin)**               | 5’-Biotin-C<sub>6</sub>-GTGTGCTTTACGCGAATGTCGTCACACGCACACGCGATCCAGCCTG-3’|
| **Y03(Ebo)**                  | TGTTGGTTATAAT-3’                                                        |
| **Y03(Sars)**                 | GCTGTGAACCAA-3’                                                         |

Note that /Phos/ represents the phosphorylation on the 5’ end of the oligonucleotide. The labeled fluorescent dye is represented by a dot with the same color. The same colored sequences represent complementary pairs.
Supplement Table 3. Oligonucleotide sequences of pathogen DNA and DNA building blocks for target-driven polymerization (Ban = Anthrax, Ebo = Ebola, Sars = SARS)

| Strand  | Sequence                                           |
|---------|----------------------------------------------------|
| Target (Ban) DNA | 5' - GGATTATTGTTAAATATTGATAAGGAT -3' |
| Target (Ebo) DNA | 5' - CATGTCAGTGATTATTATAACCCACCA -3' |
| Target (Sars) DNA | 5' - ATAATACTGCGTCTTGGTTCACAGC -3' |
| Unrelated DNA | 5' - AGATGCAATAGTAATCAGGTAGAGACG -3' |

Y │ D   │ N   │ A (Probe1)  
Y01(Ban) | 5' - TTAACAATAATCCGCCACTGGATCCGCATGAGGTAGGACGACATTCGCCGTAAGCACAC -3' |
| Y01(Ebo) | 5' - AATCACTGACATG |
| Y01(Sars) | 5' - GACGCAGTATTAT |

Y │ D   │ N   │ A (Biotin)  
Y02 | 5' - Biotin-C6-GTGTGCTTACGGCGAATGTCGTCACAGCACCGAATCAGCCTGTTCGA-3' |

Y │ D   │ N   │ A (NH2)  
Y03 | 5' - GCCACTGGATCCGCATGAGGTAGGACGACATTCGCCGTAAGCACAC-3' |

To experimentally decode the color signal of polymeric spheres, the signal intensity is counted as photon counts per pixel area with a pixel in the microscope image.

Supplement Table 4. Pre-assigned fluorescence code library of nano-architectures

| Polymeric sphere | ABC monomer 1 | ABC monomer 2 | Target DNA       |
|------------------|---------------|---------------|------------------|
| 1G3R             | 2R            | 1G1R          | Bacillus anthracis |
| 4G0R             | 2G            | 2G            | Ebola virus      |
| 2G2R             | 1G1R          | 1G1R          | SARS Coronavirus |
| (No polymer formation) | 1G1R | 1G1R | Unrelated DNA |

To experimentally decode the color signal of polymeric spheres, the signal intensity is counted as photon counts per pixel area with a pixel in the microscope image.
**Supplement Table 5.** The coefficient of variation (CV) based on the concentration of the target pathogen DNA

| Target concentration | CV (%) |
|----------------------|--------|
| 1nM                  | 7.6    |
| 250pM                | 5.6    |
| 50pM                 | 6.8    |
| 12.5pM               | 7.4    |
| 2.5pM                | 10.4   |
| 250fM                | 10.1   |
| 100fM                | 10.3   |
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