SI Appendix

DNA Origami Protection and Molecular Interfacing through Engineered Sequence-Defined Peptoids

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Materials
All single-stranded DNA (ssDNA) sequences were purchased from Integrated DNA Technologies and the M13mp18 ssDNA scaffold was purchased from Bayou Biolabs. 2-(2-(2-methoxyethoxy)ethoxy)ethanamine was purchased from Aurum Pharmatech. Rink Amide resin, 2-methoxyethylamine, propargylamine, magnesium chloride (MgCl\textsubscript{2}), copper (II) sulphate

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(CuSO₄), aminoguanidine hydrochloride, DNase I, Doxorubicin, agarose (medium EEO), bovine serum albumin, trypsin, azide-fluor 488 and phosphate buffer saline (PBS) were purchased from Sigma Aldrich. The 10X tris-acetate-ethylenediaminetetraacetic acid (EDTA) (TAE) and 10X trisborate-EDTA (TBE) buffers, 10X BlueJuice gel loading buffer, SYBR Green I (10,000x) SYBR Gold (10,000x) dyes and NHS-fluorescein were purchased from ThermoFisher Scientific. Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) was purchased from Lumiprobe. Sodium-L-ascorbate was purchased from Fluka. Gold nanoparticles (10 nm) were purchased from BBI Solutions. Carbon grid (200 mesh copper), immunogold reagent (6 nm Au) and uranyl acetate solution were purchased from Electron Microscopy Sciences. Milli-Q (18 MΩ · cm) was used for all the experiments.

Method

Preparation of octahedra-shaped DNA origamis (OCs). OCs were folded by mixing 20 nM of M13mp18 scaffold DNA and 100 nM of each staple oligonucleotides in TAE (1X) buffer containing 12.5 mM MgCl₂. The mixed solution was then cooled down from 90 °C to room temperature over 20 h to obtain the target OC structure. After synthesis, OCs were purified using the Amicon 100k centrifugal filter units (Millipore Sigma) and centrifuged at 400 g and at 4 °C. The purification process was repeated 6 times by adding fresh TAE (1X) buffer containing 12.5 mM MgCl₂ in each cycle.

OC sequence

OC-staple-1 TCAAAGCGAACCAGACCCTTATTATATAGTC
OC-staple-2 GCTTGTAGAGACTAAAGAAGCGAACCCTTATTATAGTC
OC-staple-3 GTAAATCGCTGCTATTGAAATAACTCAAGAA
OC-staple-4 AAGCCTTAATCAAGACTTGCAGGAGCAAT
OC-staple-5 ATTTTAAGAAGTGGCTTGAAATTACAGTG
OC-staple-6 GTAAAATTCGATTAAACTCAAGACTTGCA
OC-staple-7 AGCACCATTACCATTACGCAAATGACCGGA
OC-staple-8 ATTGGCAGATATTTTAAACAGATTGTGG
OC-staple-9 TAACCTGTATGCTATTTCCTCAGGACTTTC
OC-staple-10 GTCACCGGGGTAATTGGAACCAACAAAAATAG
OC-staple-11 CTCCAGCCAGCTTTCCCTCAGGACGTG
OC-staple-12 GTCCACTATTAAAGAACCAGTTTGGTTC
OC-staple-50 GTTTGCTATTCCACAGGCAGTCAGACGCCACCACACCACCC
OC-staple-51 CGCGAGCTTAGTTTTTCCCAATTCAGGCTAAAGGCTT
OC-staple-52 AGAAGCAACCAAGCGTCTATTGCCTTCCACAGGCTAAAGGCTT
OC-staple-53 ATTAATGTAAGGACGAGAGCCAAAAGCTAATAGGTG
OC-staple-54 CAGTGCCTACTGGGATTTACCGGCCTCAAAAGCTT
OC-staple-55 ATAAGGGGCGGTCAGGATTTAGGATAACGCCGGGACTCC
OC-staple-56 TGTAAAGCTT
OC-staple-57 CAGCTACG
OC-staple-58 CGGAATAACTC
OC-staple-59 GAGCTC
OC-staple-60 AGAAGCAACCAAGCGGCTCAC
OC-staple-61 CAGCGAACATTAAAAGAGAGTACCTTTACTGAATATAATGAA
OC-staple-62 GGACGTTTAACTCGAGAACACACCACTAATAGCA
OC-staple-63 AAAAGCCGAAAGACCGTTCAGGCCACCACACCACCC
OC-staple-64 GACCTCGTGAACCGGCTAC
OC-staple-65 AATTATTGTTCATGCTTACGTAC
OC-staple-66 AAGTTTCAGACAGCGGATCGTCAC
OC-staple-67 GATTATAATTTCATGCCTTTAGCGTCAGATAGCACGGAAAC
OC-staple-68 ACAAACCGAACATCATTTG
OC-staple-69 CATAACCAAATCAACAGGCCTCACCATAGCTA
OC-staple-70 CAGCGAACATTAAAAGAGAGTACCTTTACTGAATATAATGAA
OC-staple-71 CCTACCAACAGTAATTTATCTCTGACGAAACAGCCATATGA
OC-staple-72 GATTAACCGAACACACCGCCAGTTCAAAATTTACCACACGCACAG
OC-staple-73 AGTAGATTTGAAGAAATCATATTGTCATAGGCGGAAAGGCTAAAGT
OC-staple-74 TAGAATCCATAAATCATTGTCTGGAATATTTATCTCTCGGCTTAGT
OC-staple-75 AAAAGCCGAAAGACCGTTCAGGCCACCACACCACCC
OC-staple-76 CAAAAGGAAAGGACACAGGTTTTGAGCGAATCATCATATTCC
OC-staple-77 GAAATCGATAACCGGATACGGGATAGTTGATAGCCTACAGCG
OC-staple-78 TGAATATTATCAAAATATAATTGAAGGGTTAATATTATATTATCCAA
OC-staple-79 GAGGAAGCAGGATCGGCTAC
OC-staple-80 GGTGTATTTTCCACAGACACGCCCCTACCTCGTCAAGGATAG
OC-staple-81 CAAGCCCGACCCCTAGGACAGGAGATCTCTAAAGGTT
OC-staple-82 TGTAGATATTAGCGGCGATCGGCGGCGCATCTCTCTCG
OC-staple-83 CATCCTATTCAATTGAAGTTAAATGTAACAGGAAAGCAGCAGGT
OC-staple-84 CAGCTCATAAAGCGTACCACCGTTGATTGTCATGCTAGT
OC-staple-85 CATGTCACACCGAACATTAAATATTGGAAGCAGCAATTGCGTAT
OC-staple-86 AGCGTCACGTATAAGAATTGAGTTAAGGCCCTTTTAAAGAAAG
Note: To encapsulate Au NPs and BSA inside the OCs, add ‘ATCCATCACTTCATACCTACGTTGTTTT’ in front of the red-marked sequences.
**Peptoid synthesis and purification.** Peptoids were synthesized by solid phase peptoid synthesis on the Symphony X (Gyros Protein Technologies) and the synthesis procedure followed the method previously described (1). All solvents and reagents were purchased from commercial sources and used without further purification. Briefly, 200 mg of Rink amide polystyrene resin (0.61 mmol/g, Novabiochem) was swelled in DMF and the Fmoc group was deprotected in 20% (v/v) 4-methylpiperidine/DMF prior to the submonomer cycle. The acylation step was performed on the amino resin by the addition of 1.0 mL of 0.8 M bromoacetic acid and 1.0 mL of 0.8 M N,N'-diisopropylcarbodiimide in DMF and agitated with N₂ for 20 min. Displacement of the bromide with the submonomers was performed by adding 1 M solution of the primary amine in N-methyl-2-pyrrolidone followed by agitation for 30 min. The peptoids were cleaved from the resin by the addition of trifluoroacetic acid/triisopropylsilane/deionized water (95:2.5:2.5, v/v/v) solution for ~2 h, followed by evaporation using the Biotage V10 Evapoator and precipitation with an excess of cold diethyl ether. The crude peptoids were re-dispersed in deionized water followed by lyophilization. Finally, the peptoids were purified by reverse-phase high-performance liquid chromatography (HPLC, Shimadzu) using a linear gradient of 5-95% acetonitrile in water with 0.1% TFA. The Phenomenex C18 Gemini NX column was 150 × 21.2 mm and had a 5 µm pore size and 100 Å particle size.

**Preparation of peptoid-coated OCs (OC/peptoid).** The synthesized peptoids were dissolved in deionized water at a concentration of 10 mM and stored at 4 °C. For all experiments, peptoids at different concentrations based on the target ratios between the free peptoid amine and DNA phosphate (N/P) were mixed with OCs (5–45 nM) and incubated overnight (≥12 h) at 4 °C.

**Negative-stained TEM imaging.** The OC/peptoid structures were typically confirmed using negative-stained TEM imaging. In brief, 5 µL of the OC/peptoid (5–10 nM) solution was dropped onto a carbon film grid for 1–3 min and the residual liquid was removed with a piece of filter paper. After that, the grid was washed with 5 µL of deionized water followed by staining with 5 µL of 2 wt % uranyl acetate for 15 sec. The excess liquid was removed with filter papers. TEM imaging was performed on a JEOL 1400 TEM with an acceleration voltage of 120 kV.
**Agarose gel electrophoresis (AGE).** In a typical experiment, agarose (0.8 wt%) was prepared in TBE (1X) buffer containing 12.5 mM MgCl$_2$ and 1X SYBR Gold dye. OCs (4.3 nM, ~40 µL) were mixed with 1X BlueJuice gel loading buffer (Thermo Fisher Scientific) prior to loading into the gel. The gel electrophoresis was performed at 60 V on ice to prevent heating damage.

**SYBR Green I (SG) fluorescence assay of OCs.** OCs (1 nM) were mixed with peptoids in PBS (1X) buffer at an N/P of 0.125 and incubated overnight at room temperature. Prior to measuring fluorescence, 0.8X SG was added to the OC/peptoid solutions for 2 h at room temperature. The fluorescence signal was recorded from 37 °C to 85 °C at a step of 0.2 °C/min by LightCycler 480 (Roche).

**SYBR Green I (SG) fluorescence of duplex DNA (dsDNA).** A 15-bp dsDNA was designed with a sequence of 5’-ATTACCGTATAGCAT-3’ with a complementary sequence of 5’-ATGCTATACGGTAAT-3’. The dsDNA (500 µM) was formed in PBS (1X) buffer and cooled from 70 °C to room temperature over 11 h. Next, the dsDNA (100 nM) solution was mixed with different peptoid solutions at varied concentrations in PBS overnight at room temperature. Prior to measuring fluorescence, SG (1X) was added to the dsDNA/peptoid mixtures for 2 h at room temperature. The fluorescence signal was recorded from 37 °C to 85 °C at a step of 0.03 °C/sec by LightCycler 480 (Roche). The concentrations of only peptoids in solution were the same as those in the N/P of 8, which were 3.43 µM for PE1, PE3, PE4, PE5 and 1.85 µM for PE2, respectively.

**Molecular dynamics (MD) simulation of peptoid-DNA interactions.** All-atom MD simulations were performed to investigate the mechanisms of interactions and binding of brush-type PE1 and block-type PE4 peptoids with DNA in explicit solvent. The 15-bp dsDNA (5’-ATT ACC GTA TAG CAT-3’) structure was generated using the nab program available through AMBER18 (2). The peptoids were built using the Dassault Systèmes BIOVIA Materials Studio package (3), and their atomic charges were calculated with B3LYP/6-31G* level of theory using the RESP procedure (4) and Gaussian16 (5). The force field parameters for the peptoids, except the atomic charges, were generated using antechamber (6) and gaff (7). The ParmBSC1 force field (8) was employed to model the dsDNA. The molecular structures of the dsDNA, PE1 and PE4 peptoids are shown in Fig. S6. Each peptoid was placed in a triclinic simulation cell ~11 Å away from the
pre-equilibrated dsDNA structure to avoid any initial interactions. To solvate the systems, explicit water (TIP3P) molecules were added to the simulation cell to a corresponding water density of \( \sim 1.0 \text{ g/cm}^3 \). Counter-ions (Na\(^+\)) were included to neutralize the net negative charge of the system, and 150 mM NaCl to represent physiological environment.

All simulations were performed using the GROMACS simulations package (9). Particle mesh Ewald (PME) (10) electrostatic summation was truncated at 11 Å, while a force-switched cut-off starting at 9 Å and ending at 10 Å was used for the Lennard-Jones non-bonded interactions. Cubic interpolation was used with 10 Å Fourier spacing and an Ewald tolerance of \( 10^{-6} \). The MD simulations were performed in the NPT (isothermal-isobaric) ensemble. The temperature of the system was coupled at 300 K using the Nosè-Hoover thermostat (11) and pressure was maintained at 1 bar using the Parinello-Rahman barostat (12). An integration time-step of 2 fs was applied with all hydrogen bond lengths constrained using the LINCS algorithm (13). Each system was energy minimized using the steepest descent approach with a convergence criteria of 500 kJ mol\(^{-1}\) nm\(^{-1}\) to remove any steric clashes. Following the energy minimization, a 200 ps of MD simulation was performed with position restraints applied to the solute to allow the solvent to equilibrate. Initial unrestrained MD simulations were performed on the dsDNA for 50 ns to equilibrate its structure. A peptoid was then added to the simulation box sufficiently apart to prevent any initial interactions with the dsDNA. To further emulate spontaneous DNA-peptoid binding and explore wider conformational space four different starting arrangements were simulated, where the peptoid was positioned at 0°, 90°, 180° and 270° relative to the dsDNA. A 100 ns unrestrained MD was conducted for each peptoid arrangement where the peptoid was allowed to spontaneously adsorb onto the DNA from solution. The simulation trajectories from all four starting arrangements per dsDNA/peptoid complex were concatenated for further analysis. A total of 800 ns of simulation data was collected.

The different modes of interaction between the peptoids and dsDNA were investigated using contact analysis. The total time each peptoid was in contact with the dsDNA was determined, and these frames were extracted for further analysis. A contact was defined when any peptoid atom was within 4 Å of any DNA atom. The number of positively charged (Nae) residues and ethylene glycol (Nte) chains interacting with DNA and their contact stabilities was calculated. The preference of binding to specific structural features of DNA was also determined by calculating the total time each peptoid was interacting exclusively with the major or minor groove, or with
both simultaneously (atomic definition for the DNA grooves is provided in the caption of Table S1). The average contact area between each peptoid and DNA was also determined. The average solvent accessible surface area of each peptoid while bound to dsDNA was calculated using a probe radius of 1.4 Å (water) and Lennard-Jones hard-shell radii for each atom to define the surface of the peptoid. The elongation or compactness of the peptoid was estimated by measuring the average distance between the outermost nitrogen atoms of the peptoid backbone. Visualization of the trajectories and analysis was performed using the VMD software (14).

**Magnesium depletion assays.** Bare OCs and OC/peptoid were diluted in TAE (1X) buffer, PBS (1X) buffer, Roswell Park Memorial Institute 1640 medium (RPMI, Thermo Fisher Scientific) and the Dulbecco's Modified Eagle Medium (DMEM, Gibco) such that the final concentrations of origami and MgCl₂ were 4.3 nM and 1.25 mM, respectively. The samples were incubated at 4 °C (TAE and PBS) or 37 °C (RPMI and DMEM) for 24 h and characterized by AGE and negative-stained TEM imaging. In all figures of AGE analyses, label “+” represents the final MgCl₂ concentration of 12.5 mM; label “–” represents depleted Mg²⁺ and the final MgCl₂ concentration was 1.25 mM. To prepare samples for TEM imaging, OCs were extracted from the agarose gels using the Freeze 'N Squeeze™ DNA Gel Extraction Spin Columns (Bio Rad) and centrifuged at 1000 rcf for 3 min at room temperature.

**Dynamic light scattering (DLS).** In Fig. 4E, bare OCs (4 nM) and PE2-coated OCs (OC/PE2, 4 nM, N/P: 0.5) in TAE 1X buffer containing 12.5 mM MgCl₂ were incubated with EDTA (10 mM) for ~30 min prior to DLS measurement. In Fig. S21, bare (4 nM) and OC/PE2 solutions (4 nM, N/P: 0.5) were incubated with DNase I of 15 and 20 µg/mL at 37 °C for 30 min prior to measurement. The concentration of MgCl₂ in the TAE buffer was 12.5 mM. The samples were measured 3 times with Zetasizer Nano Z (Malvern Panalytical) with an equilibrium time of 120 sec.

**Small-angle X-ray scattering (SAXS).** Solution scattering data was collected at the Life Sciences X-ray Scattering beamline (LiX) at NSLSII, Brookhaven National Laboratory, Upton, NY. LiX utilizes an undulator source and a Si(111) monochromator. KB mirrors focus the beam on a secondary source and X-ray energy was 12 keV with a beamsize of ~400um. An in-house
solution scattering box houses a movable 3 channel flow cell such that proteins in solution flow through the beam during collection. Data was collected on 3 Pilatus detectors (SAXS: Pilatus 1M, 2 offset WAXS detectors: Pilatus 300K) (15). The data was merged, averaged, subtracted and packed into HDF5 format using our in-house py4xs software (16), with data visualization in a jupyter notebook. Bare OCs (28 nM) and OC/PE2 (28 nM, N/P: 0.5) in TAE 1X buffer containing 12.5 mM MgCl₂ were mixed with EDTA (10 mM) and immediately loaded to the SAXS flow cell. The total processing time prior to measurement is ~20–30 min. For each sample exposed to the X-ray beam, five frames, with an exposure time of 1 sec was collected and processed using the py4xs software. TAE 1X buffer containing 12.5 mM MgCl₂ was used as reference and was subtracted from the samples.

**Nuclease degradation assays.** Bare OCs (4.3 nM) and OC/peptoid (4.3 nM, N/P: 0.5) were mixed with different concentrations of deoxyribonuclease I (DNase I) in TAE (1X) buffer containing 12.5 mM MgCl₂. The samples were incubated at 37 °C for 30 min at 650 rpm on a thermal cycler (Eppendorf) and characterized using AGE and negative-stained TEM imaging.

**Encapsulation of 10 nm gold nanoparticles (Au NPs) in OCs.**

**Peptide synthesis.**
Solid-phase peptide synthesis was performed to synthesize the azido peptide (CALDDK(N₃)) for Au NP functionalization. Briefly, Rink Amide resins and the protected amino acids were added to the growing peptide chain with the activating reagent 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethylyuronium hexafluorophosphate (HBTU). Following addition of the submonomers, the Fmoc group was removed under 20% piperidine in DMF deprotection conditions. The peptides were cleaved by trifluoroacetic acid/triisopropylsilane/deionized water (95:2.5:2.5, v/v/v). The crude peptide was precipitated by cold ether several times and lyophilized. The as-synthesized peptides were purified by reverse-phase high-performance liquid chromatography (HPLC, Shimadzu). The Phenomenex C18 Gemini NX column was 150 × 21.2 mm and had a 5 µm pore size and 100 Å particle size.

**Au NP functionalization.**
First, 990 µL of 10 nm Au NPs (9.5 nM, BBI solutions) were mixed with 10 µL of peptide CALDDK(N₃) (pep, 1 mM) overnight at room temperature (~12 h). The solution was washed 3
times with 10 mM phosphate buffer (pH 7.4) by centrifugation at 10,000 rcf and at 4 °C. Next, DBCO-modified single-stranded DNA (ssDNA, 5’-TATGAAGTGATGGATGAT/3’DBCO/) was added to the Au NP-pep solution at concentration ratio of 300:1 in 10 mM phosphate buffer (pH 7.4) and incubated at least 4 h at room temperature. A final concentration of 100 mM of NaCl was added to the Au NP-solution and left incubating overnight. The solution was washed 3 times with 10 mM phosphate (pH 7.4) containing 100 mM NaCl by centrifugation at 10,000 rcf and at 4 °C. UV-vis spectroscopy (Lambda 25, PerkinElmer) was used to calculate the final Au NP-pep-ssDNA concentration.

**Au NP-pep-ssDNA encapsulation in OCs.**

OCs (40 nM) were mixed with the Au NP-pep-ssDNA (48 nM) and cooled from 50 °C to room temperature over a time course of 40 h.

**Doxorubicin (Dox) release from bare OCs and OC/peptoid.** OCs (10 nM) were incubated with Doxorubicin (Dox, 0.1 mM) at room temperature for 24 h and purified 3 times with TAE (1X) buffer containing 12.5 mM MgCl₂ using a 100 kDa filter and centrifuged at 400 rcf and at 15 °C. OCs were concentrated to 50 nM in the final centrifugation. OC/PE2 were prepared by mixing PE2 (27.7 µM) with Dox-loaded OCs (50 nM) overnight (~12 h) at room temperature. Dox-loaded bare OCs and OC/PE2 were diluted in PBS (1X) at pH 7 or 5.5 and incubated at 37 °C for 48 h, followed by centrifugation with 50 kDa filters at 1000 rcf for 30 min at room temperature, of which the supernatant solution was collected. Fluorescence spectra were measured in a 384 well plate (Corning) using the Spark microplate reader (Tecan). The λex and λem were 485 nm and 515–800 nm, respectively with a step size of 2 nm.

**Encapsulation of fluorescein-modified bovine serum albumin (BSA) in OCs.** First, NHS-fluorescein (240 µM) and NHS-PEG-azide (60 µM) was mixed with BSA (10 µM) in PBS (1X) buffer for 3 h at room temperature. The solution was purified 8 times with PBS using a 50 kDa filter and centrifuged at 3000 rcf and at 4 °C. Next, the DBCO-modified single-stranded DNA (ssHy, 5’-TATGAAGTGATGGATGAT/3’DBCO/, 250 µM) was mixed with the BSA solution (10 µM) overnight (~12 h) at 4 °C. The solution was purified 8 times with PBS using a 50 kDa filter and centrifuged at 3000 rcf at 4 °C. Finally, the surface modified BSA (200 nM) was added to the OCs (40 nM) and the solution was cooled down from 45 °C to room temperature over a time course
of 60 h. The solution was purified 2 times with TAE (1X) buffer containing 12.5 mM MgCl$_2$ using a 100 kDa filter and centrifuged at 400 rcf and at 4 °C.

**Fluorescence assay for tryptic digestion of BSA.** (1) The fluorescein modified BSA (80 nM) solution without OCs was incubated at 37 °C in the presence or absence of trypsin (50 nM) overnight ~12 h prior to fluorescence measurement. Fluorescence spectra were measured with excitation and emission wavelengths of 490 nm and 510-750 nm, respectively and a step size of 2 nm. (2) The fluorescein modified BSA encapsulated OCs (20 nM) were incubated at 37 °C in the presence or absence of trypsin (50 nM) in a 384 well plate. The fluorescence kinetics were measured at $\lambda_{ex}$ and $\lambda_{em}$ of 490 nm and 525 nm, respectively and time interval of 3 min. Fluorescence spectra were measured after 15 h at excitation and emission wavelengths of 490 nm and 510–800 nm, respectively and a step size of 2 nm. TAE (1X) buffer containing 12.5 mM MgCl$_2$ was used for both experiments.

**Surface conjugation of peptoid-protected OCs with Alexa Fluor 488.** Copper-catalyzed click chemistry was performed by adapting reported procedures (17). Briefly, alkyne-modified peptoids (PE8 and PE9, 20 µM) were mixed with azide-modified Alexa Fluor 488 (60 µM), 0.1 mM CuSO$_4$, 0.5 mM THPTA, 5 mM aminoguanidine and 5 mM sodium ascorbate in 100 mM sodium phosphate buffer (pH= 7.4) for 2 h at room temperature. The peptoid-fluorophore (PE8-FL and PE9-FL) conjugates were dialyzed against deionized water with 0.2 mM EDTA for 12 h at room temperature using a 3,500 Da membrane, followed by dialysis against deionized water for 24 h at room temperature.

**Synthesis of Trastuzumab-azide** Trastuzumab expressed with two heavy chain C-terminal formylglycine residues (SMARTag antibody CT) was a gift from David Rabuka (Catalent) (18). A small molecule azide linker with a hydrazino moiety was synthesized in 9 steps as described previously (17, 18). The Trastuzumab-aldehyde was reacted with the azide via the hydrazino-iso-Pictet Spengler ligation as described previously (20). Briefly, Trastuzumab with C-terminal heavy-chain formylglycine (fGly) residues was buffer exchanged via PD-10 column (GE Life Sciences, 17085101) into 50 mM sodium citrate (Millipore Sigma C8532). To fGly-modified Trastuzumab (182.7 nmol, 2.19 mL, 1 equiv.) in sodium citrate buffer was added the azide
molecule (4 µmol, 135 µL, 21.9 equiv.) freshly dissolved in DMSO in a falcon tube. The falcon tube was closed after flushing with argon gas, and the reaction was mixed 250 rpm in the dark at 37 °C for 22 h. Following this, the reaction was buffer exchanged via PD-10 desalting columns into PBS (Corning, 21-040-CM), with ~90% recovery.

Trastuzumab-azide was analyzed by mass spectrometry to confirm the azide addition, with no detected unmodified Trastuzumab-azide remaining. Trastuzumab-azide (20 µL in PBS was treated with 0.75 µL PNGaseF (NEB, P0704S) at 37 °C overnight in an eppendorf tube. After 16 h, the DTT was added (30 mM, 0.6 µL from frozen stock solution in water) (Thermo Fisher Scientific, 15508013), and the antibody was heated at 65 °C for 5 min using a Thermomixer. Antibody was placed on ice and analyzed in the same day at the SUMS facility at Stanford University by ESI-LC/MS on an Agilent 1260 HPLC and Bruker MicroTOF-Q II time-of-flight mass spectrometer. A Waters BioResolve RP mAb Polyphenyl 450Å 2.7 µm 100 x 2.1 mm column was maintained at 50 °C. Five microliters of reduced, de-glycosylated antibody conjugate were injected at a flow rate of 0.3 mL/min at 95% solvent A (0.05% trifluoroacetic acid in water) and 5% solvent B (0.1% formic acid in acetonitrile). This was held for 1.5 min, then ramped to 35% B at 2 min, 46% B at 10 min, and 95% B at 11 min, which was held for 1 min. Data was collected in full scan MS mode with a mass range of 400–4000 Da and Collision RF setting equal to 800 V.

**Surface conjugation of peptoid-protected OCs with Trastuzumab.** Copper-catalyzed click chemistry was performed by adapting reported procedures (17). Briefly, alkyne-modified peptoids (PE8 and PE9, 30 µM) were mixed with azide-modified Trastuzumab (10 µM), 0.1 mM CuSO4, 0.5 mM THPTA, 5 mM aminoguanidine and 5 mM sodium ascorbate in 100 mM sodium phosphate buffer (pH= 7.4) for 2 h at room temperature. The peptoid-Trastuzumab (PE8-Tz and PE9-Tz) conjugates were dialyzed against PBS (1X) buffer with 0.2 mM EDTA for 12 h at 4 °C using a 100-500 Da membrane for 2 times, followed by dialysis against PBS (1X) buffer at 4 °C for 24 h.
Fig. S1. Mass spectrometry of peptoids PE1–9 prepared by solid phase peptoid synthesis.
**Fig. S2.** Real-time SYBR Green I (SG) fluorescence assay of the 15-bp dsDNA in the presence of peptoids (A, B) PE1, (C, D) PE3, (E, F) PE4 and (G, H) PE5 at different N/P. Derivatives of the fluorescence intensities were plotted against the increasing temperature.
Fig. S3. Real-time SG fluorescence assay of peptoid only. (A) Fluorescence intensities and (B) derivatives of the fluorescence intensities were plotted against the increasing temperature.

Fig. S4. Fluorescence spectra of the 15-bp dsDNA in the presence of SG (1X) and PE2 at different N/P. Sample preparation and fluorescence measurements were performed at room temperature ($\lambda_{ex}=495$ nm and $\lambda_{em}=510–650$ nm). The concentration of PE2 only in solution was the same as that of N/P of 8, which was 1.85 $\mu$M.
**Fig. S5.** AGE shows the electrophoretic shift of dsDNA/peptoid complexes. (A) dsDNA/peptoid of 0.5; (B) dsDNA/PE2 at different N/P.

**Fig. S6.** Atomistic structures of the peptoid and dsDNA models used in the MD simulations. The peptoids (A) PE1 and (B) PE4 are showing the peptoid backbone in blue, charged (Nae) residues in red and ethylene glycols (Nte) in green. (C) Molecular and (D) cartoon representation of the dsDNA model are showing the DNA backbone in grey, and the bases colored individually with cytosine in red, guanine in green, thymine in yellow and adenine in blue.
Fig. S7. TEM images show OC/peptoid at N/P of 0.5, 0.25 and 0.1 (scale bars: 200 nm). The insets show magnified images of the OC structures (scale bars: 100 nm).
Fig. S8. (A) Chemical structure of the Nae$_6$–Nte$_{16}$ peptoid and (B) TEM images of Nae$_6$–Nte$_{16}$ OC/peptoid at N/P of 0.5 (scale bar: 200 nm). The inset shows magnified image of the OC structures (scale bar: 100 nm).

Fig. S9. Real-time SG fluorescence assay of bare OCs and OC/peptoid (1 nM, N/P: 0.125) in PBS buffer. Derivatives of the fluorescence intensities were normalized to 0–1 range and plotted against the increasing temperature.
**Fig. S10.** Top: Molecular structure of PE6 ((Nme–Nte)_{12}). Bottom: TEM images of OC/PE6 structures at N/P of (A) 2, (B) 1 and (C) 0.5 (scale bars: 200 nm). The insets show magnified images of the OC structures (scale bars: 100 nm).

**Fig. S11.** Top: molecular structure of PE7 (Nme_{6}–Nte_{12}). Bottom: TEM images of OC/PE7 at N/P of (A) 2, (B) 1 and (C) 0.5 (scale bars: 200 nm). The insets show magnified images of the OC structures (scale bars: 100 nm).
Fig. S12. AGE showing the electrophoretic shift of OC/peptoid (4.3 nM). Lanes 1–3: N/P of 0.5, 0.25, and 0.1, respectively; Lane 4: peptoid/OC= 1; and Lane 5: OC only.

Fig. S13. AGE of OC/peptoid in TAE buffer. The final concentration of MgCl₂ was 1.25 mM. The result was used in the calculation of electrophoretic shift in Fig. 4C.
**Fig. S14.** TEM images of (A) bare OCs and OC/PE2 at (B) N/P of 0.1 and (C) N/P of 0.5. The final concentration of MgCl$_2$ in TAE buffer was 1.25 mM. TEM samples were extracted from the agarose gels (scale bars: 200 nm). The insets show magnified images of the OC structures (scale bars: 100 nm).

**Fig. S15.** TEM images of peptoid (PE1, PE3, PE4 and PE5)-coated OCs in TAE buffer. The final concentrations of MgCl$_2$ were 12.5 mM (TAE-Mg$^{2+}$) and 1.25 mM (TAE low Mg$^{2+}$). TEM
samples were extracted from the agarose gels (scale bars: 200 nm). The insets show magnified images of the OC structures (scale bars: 100 nm).

Fig. S16. TEM images of OCs in the presence of different amounts of EDTA: (A) 12.5 mM, (B) 10 mM, (C) 6.25 mM, (D) 3.5 mM and (E) 0 mM. The concentration of MgCl₂ in TAE buffer was 12.5 mM. EDTA was added to the OC solution and left undisturbed for ~4 h at room temperature prior to TEM samples preparation (scale bars: 200 nm). The insets show magnified images of the OC structures (scale bars: 100 nm).
Fig.S17. Kratky analysis (21) of SAXS data in Fig. 4F and G. Bare OCs appeared to be more flexible upon treatment with EDTA and did not plateau at higher $q$ compared to the OC/PE2.
**Fig. S18.** Left: AGE of bare OCs and OC/peptoid in PBS buffer. The final concentration of MgCl$_2$ was 1.25 mM. Right: TEM samples were extracted from the agarose gels (bands a–f). Scale bars: 200 nm. The insets show magnified images of the OC structures (scale bars: 100 nm).

**Fig. S19.** AGE of OCs in the presence of different concentrations of deoxyribonuclease I (DNase I). Degradation of OC nanostructures by DNase I was shown by the electrophoretic shift toward the end of lower molecular weight and the presence of new bands representing degraded OCs.
Fig. S20. TEM images of bare OCs and OC/peptoid in the absence (left) and presence of DNase I (15 µg/mL, right). The samples were extracted from agarose gels. The concentration of MgCl₂ in TAE buffer was 12.5 mM (scale bars: 200 nm). The insets show magnified images of the OC
structures (scale bars: 100 nm). Among the peptoid sequences, only OC/PE2 showed protection of the OC nanostructures.

**Fig. S21.** DLS of (A) bare OCs and (B) OC/PE2 (N/P: 0.5) in the presence of DNase I. The extent of size reduction represents degradation of the OC nanostructures by DNase I.

**Fig. S22.** TEM images of (A) bare OCs and (B) OC/PE2 in DMEM cell media containing FBS (0%, 5% and 10%). The final concentration of MgCl$_2$ was 1.25 mM. TEM samples were extracted from the agarose gels (scale bars: 200 nm). The insets show the magnified images of the OC structures (scale bars: 100 nm).
Fig. S23. UV-vis spectra of step-wise functionalization of 10 nm gold nanoparticles (Au NPs) with Cys-Ala-Leu-Asp-Asp-Lys(N$_3$) (pep) and followed by a DBCO-modified single-stranded DNA (ssDNA, 5’-TATGAAAGTGATGGATGAT/3DBCO/), which complemented with the eight ssDNAs located in the OCs.

Fig. S24. (A) AGE and (B) TEM images show PE2 coated and Au NP-encapsulated OCs in DMEM media containing FBS (0%, 5% and 10%). AGE was performed and imaged by white light (top) and UV light (bottom). TEM samples were extracted from the agarose gels (scale bars: 200 nm). The insets show magnified images of the OC structures (scale bars: 50 nm).
Fig. S25. (A) Fluorescence spectrum of Doxorubicin (Dox, 100 μM) in PBS buffer. Excitation and emission wavelengths were measured at 485 nm and 510–800 nm, respectively. (B) Fluorescence signals at 597 nm were plotted against Dox concentrations ([Dox]). A linear relationship between fluorescence signal and [Dox] was observed below 3.2 μM.

Fig. S26. Plot showing the total Dox release from bare OCs and OC/PE2 (n= 2). The Dox release was determined by the remaining fluorescence of OCs after incubating in PBS buffer at 37 °C for 48 h. A reduction of total Dox release from the OC/PE2 compared to bare OCs was observed at both pH 7 and 5.5.
Fig. S27. Fluorescence enhancement of fluorescein labeled BSA (80 nM) in the presence and absence of trypsin (50 nM). The concentration of MgCl₂ in TAE buffer was 12.5 mM. The solution was incubated overnight (>12 h) at 37 °C prior to fluorescence measurement (λ_ex = 490 nm and λ_em = 510–800 nm).

Fig. S28. Fluorescence spectra of fluorescein-labelled BSA encapsulated in bare OCs and OC/PE2 (20 nM) in the presence and absence of trypsin (50 nM). The concentration of MgCl₂ in TAE buffer was 12.5 mM. The mixtures were incubated at 37 °C for ~15 h prior to fluorescence measurement (λ_ex = 485 nm and λ_em = 510–800 nm).
**Fig. S29.** TEM images of OC in the presence of different trypsin concentrations: (A) 5 µM, (B) 0.5 µM, (C) 0.1 µM and (D) 0 µM (scale bars: 200 nm). The insets show magnified images of the OC structures (scale bars: 100 nm).

**Fig. S30.** Site-specific modification of Trastuzumab. Top: schematic view of the modification. Bottom: ESI-LC/MS analyses of the Trastuzumab-azide.
Table S1: Peptoid-DNA interaction characterization and properties.

| Properties                                                                 | PE1          | PE4          |
|---------------------------------------------------------------------------|--------------|--------------|
| **a** Persistent Contact (%)                                              | 98.2%        | 98.7%        |
| **b** Minor/Major groove interactions                                      |              |              |
| Minor Groove Contacts (%)                                                 | 28.5%        | 24.0%        |
| Major Groove Contacts (%)                                                 | 6.1%         | 39.6%        |
| Major and Minor Groove Contacts (%)                                        | 48.8%        | 18.5%        |
| **b** Nte/Nae residue interaction time                                     |              |              |
| Nte Contact (%)                                                           | 98.02%       | 70.39%       |
| Nte (1 residue)                                                           | 12.55%       | 30.45%       |
| Nte (2 residues)                                                          | 24.75%       | 24.94%       |
| Nte (3 residues)                                                          | 31.79%       | 11.82%       |
| Nte (4 residues)                                                          | 20.19%       | 2.90%        |
| Nte (5 residues)                                                          | 8.07%        | 0.28%        |
| Nte (6 residues)                                                          | 0.67%        | 0.00%        |
| Nae Contact Time (%)                                                       | 98.08%       | 99.90%       |
| Nae (1 residue)                                                           | 14.29%       | 0.66%        |
| Nae (2 residues)                                                           | 25.53%       | 3.87%        |
| Nae (3 residues)                                                           | 31.53%       | 12.74%       |
| Nae (4 residues)                                                           | 19.87%       | 28.43%       |
| Nae (5 residues)                                                           | 6.45%        | 35.86%       |
| Nae (6 residues)                                                           | 0.41%        | 18.33%       |
| **Average contact area (nm²)**                                            | 2.88±1.11    | 2.54±1.05    |
| **Peptoid solvent accessible surface area (nm²)**                         | 18.82±1.47   | 17.81±1.64   |
| **Average separation distance of outermost nitrogens dₙ₂₋ₙ₁₉ (nm)**        | 2.33 ± 0.6   | 2.20 ± 0.6   |
Reference:
1. J. Sun, G. M. Stone, N. P. Balsara, R. N. Zuckermann, Structure–Conductivity Relationship for Peptoid-Based PEO–Mimetic Polymer Electrolytes. *Macromolecules* **45**, 5151–5156 (2012).
2. D. M. Y. P. A. K. D.A. Case, I.Y. Ben-Shalom, S.R. Brozell, D.S. Cerutti, T.E. Cheatham, III, V.W.D. Cruzeiro, T.A. Darden, R.E. Duke, D. Ghoreishi, M.K. Gilson, H. Gohlke, A.W. Goetz, D. Greene, R Harris, N. Homeyer, S. Izadi, A. Kovalenko, T. Kurtzman, T.S. Lee, S. LeGra, AMBER 2018 (University of California, San Francisco) (2018).
3. BIOVIA DS (2018) Materials Studio (San Diego: Dassault Systèmes) (2018).
4. C. I. Bayly, P. Cieplak, W. Cornell, P. A. Kollman, A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: the RESP model. *J. Phys. Chem.* **97**, 10269–10280 (1993).
5. I. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V., Gaussian 16, Revision B.01 (Wallingford CT) (2016).
6. J. Wang, W. Wang, P. A. Kollman, D. A. Case, Automatic atom type and bond type perception in molecular mechanical calculations. *J. Mol. Graph. Model.* **25**, 247–260 (2006).
7. J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, D. A. Case, Development and testing of a general amber force field. *J. Comput. Chem.* **25**, 1157–1174 (2004).
8. I. Ivani, P. D. Dans, A. Noy, A. Pérez, I. Faustino, A. Hospital, J. Walther, P. Andrio, R. Goñi, A. Balaceanu, G. Portella, F. Battistini, J. L. Gelpí, C. González, M. Vendruscolo, C. A. Laughton, S. A. Harris, D. A. Case, M. Orozco, Parmbsc1: a refined force field for DNA simulations. *Nat. Methods*, 2015/11/16 Ed. **13**, 55–58 (2016).
9. M. J. Abraham, T. Murtola, R. Schulz, S. Páll, J. C. Smith, B. Hess, E. Lindahl, GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **1–2**, 19–25 (2015).
10. T. Darden, D. York, L. Pedersen, Particle mesh Ewald: An N·log(N) method for Ewald sums in large systems. *J. Chem. Phys.* **98**, 10089–10092 (1993).
11. S. Nosé, A molecular dynamics method for simulations in the canonical ensemble. *Mol. Phys.* **52**, 255–268 (1984).
12. M. Parrinello, A. Rahman, Polymorphic transitions in single crystals: A new molecular
dynamics method. *J. Appl. Phys.* **52**, 7182–7190 (1981).

13. B. Hess, H. Bekker, H. J. C. Berendsen, J. G. E. M. Fraaije, LINCS: A linear constraint solver for molecular simulations. *J. Comput. Chem.* **18**, 1463–1472 (1997).

14. W. Humphrey, A. Dalke, K. Schulten, VMD: Visual molecular dynamics. *J. Mol. Graph.* **14**, 33–38 (1996).

15. J. DiFabio, S. Chodankar, S. Pjerov, J. Jakoncic, M. Lucas, C. Krywka, V. Graziano1, L. Yang, The life science x-ray scattering beamline at NSLS-II. *AIP Conf. Proc.* **1741**, 30049 (2016).

16. L. Yang, Using an in-vacuum CCD detector for simultaneous small- and wide-angle scattering at beamline X9. *J. Synchrotron Radiat.* **20**, 211–218 (2013).

17. S. I. Presolski, V. P. Hong, M. G. Finn, Copper-Catalyzed Azide-Alkyne Click Chemistry for Bioconjugation. *Curr. Protoc. Chem. Biol.* **3**, 153–162 (2011).

18. P. M. Drake, A. E. Albers, J. Baker, S. Banas, R. M. Barfield, A. S. Bhat, G. W. de Hart, A. W. Garofalo, P. Holder, L. C. Jones, R. Kudirka, J. McFarland, W. Zmolek, D. Rabuka, Aldehyde Tag Coupled with HIPS Chemistry Enables the Production of ADCs Conjugated Site-Specifically to Different Antibody Regions with Distinct in Vivo Efficacy and PK Outcomes. *Bioconjug Chem* **25**, 1331–1341 (2014).

19. P. Agarwal, R. Kudirka, A. E. Albers, R. M. Barfield, G. W. de Hart, P. M. Drake, L. C. Jones, D. Rabuka, Hydrazino-Pictet-Spengler ligation as a biocompatible method for the generation of stable protein conjugates. *Bioconjug. Chem.* **24**, 846–851 (2013).

20. M. A. Gray, M. A. Stanczak, H. Xiao Han, J. F. A. Pijnenborg, N. R. Mantuano, S. A. Malaker, P. A. Weidenbacher, C. L. Miller, J. T. Tanzo, G. Ahn, E. C. Woods, H. Läubli, C. R. Bertozzi, Targeted desialylation overcomes glyco-immune checkpoints and potentiates the anticancer immune response in vivo. chemrxiv: 8187146.v2 (2019).

21. J. B. Hopkins, R. E. Gillilan, S. Skou, BioXTAS RAW: improvements to a free open-source program for small-angle X-ray scattering data reduction and analysis. *J. Appl. Crystallogr.* **50**, 1545–1553 (2017).