A logic-gated nanorobot for targeted transport of molecular payloads.

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Recent evidence suggests that spatial organization of proteins at cell-cell interfaces is an important regulatory mechanism in intercellular signal transduction [1]. Lithographic techniques have been used to pattern surfaces to examine micron-scale spatial interactions of receptors [2], while crystallographic studies have resolved angstrom-scale interactions within immune signaling complexes [3]. However, our understanding of and ability to control these mechanisms is incomplete due to the difficulty in controlling matter at scales of 1–100 nm. To address this gap, we have created devices for studying nanometer-scale spatial arrangement of effector molecules in cell-surface signal transduction.

Templated self-assembly of DNA into custom shapes has been demonstrated by annealing hundreds of short single-stranded DNA oligonucleotides with a multi-kilobase 'scaffold' DNA strand [4]. Shapes with 100 nm dimensions of can be readily designed using our CAD software. Proteins can be attached to shapes in an addressable fashion with nanometer-scale precision. By combining these techniques with relevant surface receptors and administering them to various cell types, distance-dependent multivalent binding effects on immune signaling can be systematically investigated.

DNA nanostructures can also be used to generate artificial signaling events to skew immune responses to desired outcomes. Using leukemia treatment as a proof-of-concept, we have developed a modular, targeted immunotherapeutic delivery system in the form of a DNA capsule with a diameter of 35 nm [5]. Antibodies recognizing selected targets on leukemic cell surfaces are loaded inside the capsule, which is held closed by a DNA-aptamer-based lock. When the aptamer sequence of the lock binds to its leukemic cell marker antigen, the capsule turns inside out and exposes the payload to the cell surface, inducing apoptosis. The lock mechanism appears to minimize off-target effects, and we expect that packing different therapeutics within a single capsule may reduce development of resistance. We anticipate that our design could serve as the bases for a new class of therapeutic devices for intervention in diseases such as immune deficiencies, chronic inflammation, and cancer.

1. Manz & Groves, Nat Rev Mol Cell Biol. 2010.
2. Mossman et al., Science 2005.
3. Kato et al., PNAS 2008; Deindl et al., PNAS 2010.
4. Rothemund, Nature 2006; Douglas et al., Nature 2009.
5. Douglas, Bachelet, & Church, 2011. *submitted.*
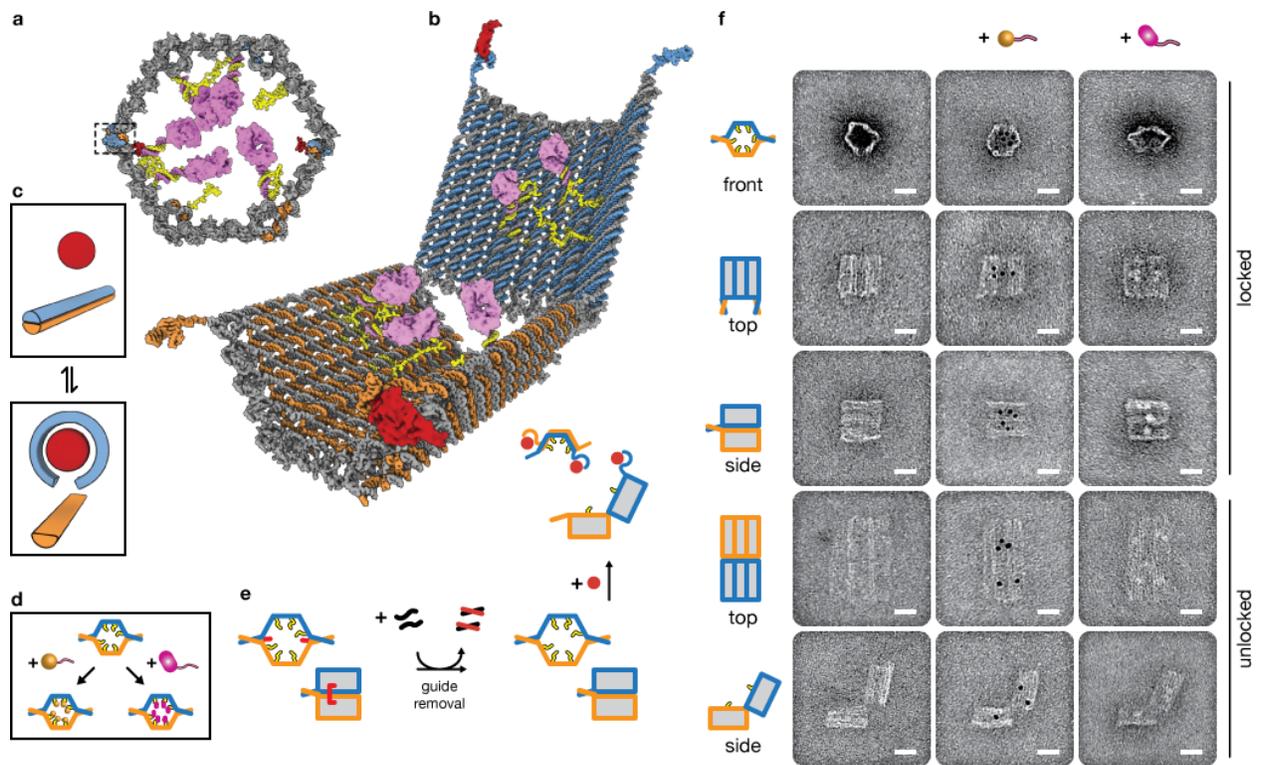
Figure 1 — Design and TEM analysis of an aptamer-gated DNA nanorobot. The device transitions from its closed state (a) to open (b) when aptamer-based locks are displaced by binding to an antigen key (c). Payloads such as gold nanoparticles and antibody fragments (d) can be loaded. Removable guide staples (e) aid in initial folding to high yield. Electron micrographs show the nanorobot with different payloads and conformations. Scale bars 20 nm.
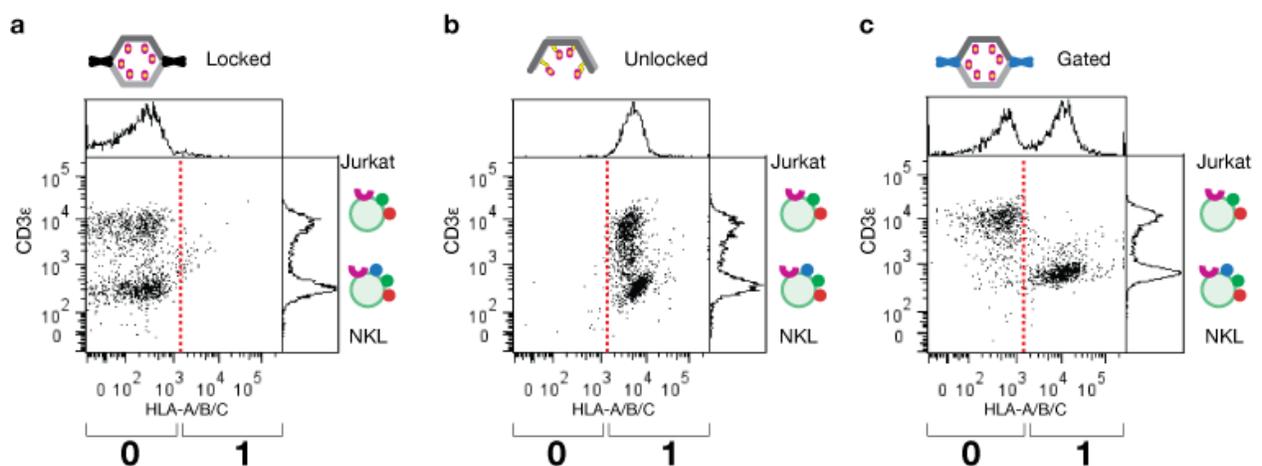
Figure 2 — Flow-cytometric analysis of nanorobot selectivity in a complex mixture. (a) NKL and Jurkat cells were mixed, labeled with FITC-anti-human CD3ε and incubated with robots loaded with APC-anti-human HLA A/B/C Fab' for 30 min. Locked robots remain inactive. (b) Unlocked robots react with both cell populations. (c), Gated robots react only with the cell population expressing the proper key.
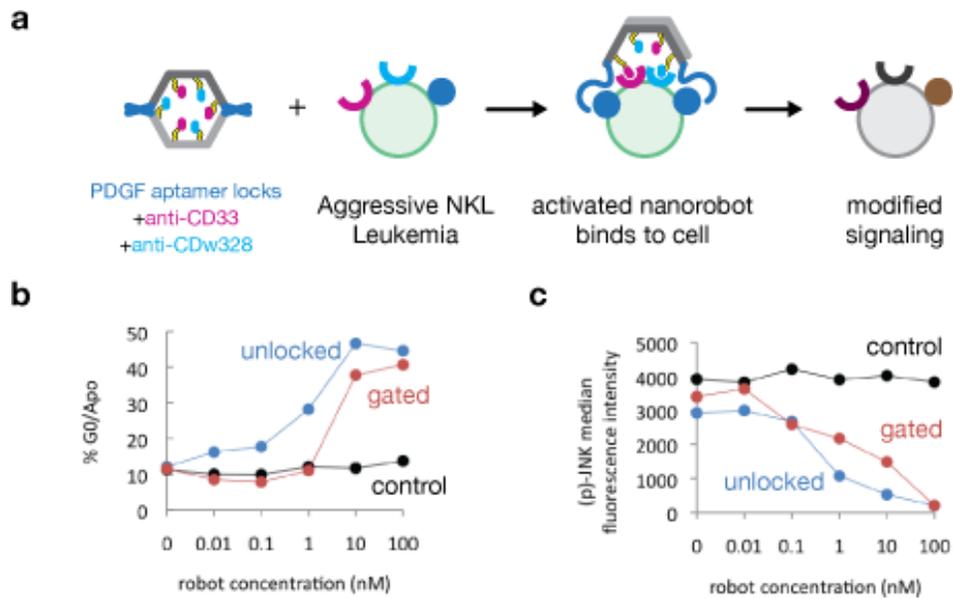
Figure 3 — Using DNA nanorobots to manipulate target cell signaling. (a) Experimental scheme. A single dose of nanorobots loaded with an equal mixture of anti-human CD33 and anti-human CDw328/Siglec-7 Fab' fragments (cyan, magenta) and gated by anti-PDGF locks (blue lines) were used to treat NKL cells at various concentrations (0–100 nM). Phosphorylation of JNK was measured after 72h by intracellular flow cytometry. (b) NKL cells treated with nanorobots were analyzed after 72h for cell cycle distribution by propidium iodide (5 μg/mL). (c) Phosphorylation level of JNK as a function of robot concentration (lowest, 0; highest, 100 nM) after 72 h.