

Platform: Biotechnology & Bioengineering

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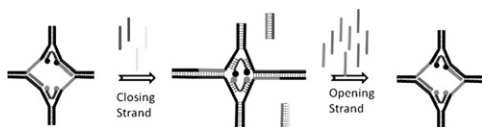
DNA Based Dual-Spring Cross Shaped Nanoactuator

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DNA is an attractive platform for nanotechnology applications because of its size, specificity, and designability. However constructing DNA-based platforms that can do work is difficult. We have developed a DNA-based cross-shaped nanoactuator system that cycles between an extended and contracted confirmation relying on strand displacement reactions. The actuator contains 4 structural strands with two unique DNA “zipper” sequences. Each zipper sequence employs traditional adenosine-thymine nucleotides as well as non-traditional inosine-cytidine nucleotides. The I-C bond consists of only 2 hydrogen bonds as opposed to the typical 3 hydrogen bonds found in G-C bonds. The actuator is extended by inserting two ssDNA which are the natural complements to the zipper sequences. The natural complements have a stronger binding affinity to one side of the zipper than both zipper strands have to each other, thus unraveling and allowing the actuator to extend. The two contraction strands contain sequences which are a natural complement to parts of the opening strand. When they bind to the extension sequences, the zippers are able to rebind and this contracts the actuator. Proper assembly and function of the devices was confirmed

using fluorescent DNA gel electrophoresis, AFM imaging, and time-lapsed fluorescence.



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Creation of a Biological Wire using Cell-Targeted Paramagnetic Beads

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Introduction: Cardiac conduction delays and blocks are associated with re-entrant arrhythmias. We sought to create a novel approach to treat such disorders by engineering biological wires designed to bridge or bypass zones of slow conduction.

Methods: Paramagnetic beads (8 μm diameter) were conjugated with an antibody specific for γ-sarcoglycan, a cardiomyocyte cell-surface antigen. Freshly-isolated neonatal rat ventricular cardiomyocytes (NRVMs) were exposed to antibody-coated beads. A biological wire (BioWire) was formed by exposing the bead-NRVM complex to a linear magnetic field. To create a model of conduction block, NRVMs were plated in monolayers and mechanically interrupted along the middle. Action potential (AP) propagation and AP duration were measured by optical mapping.

Results: Prior to bridging, the two sides of an interrupted NRVM monolayer beat independently. In order to re-establish conduction, BioWire was formed perpendicular to the axis of interruption, by placing a magnet bridging the two halves underneath the monolayer. Within one day of BioWire implantation, the two NRVM islands beat synchronously, and APs propagated from one island to the other via BioWire with a conduction velocity (CV) of 18 ± 4 cm/s. Action potential morphology and APD90 were similar in BioWire (APD90=443 ± 5ms) and the adjacent monolayers (APD90=439 ± 12ms, p=ns). BioWire was amenable to further engineering. Cardiosphere-derived cells (CDCs), which can couple to and exert anti-apoptotic effects on cardiomyocytes, were mixed with NRVMs and conjugated to the beads via CD105 (≤ 5% CDCs, BioWire-mx) in order to enhance the physical integrity of BioWire. When paced, BioWire-mx showed faster CV than that of BioWire (17 ± 6 vs 11 ± 1 cm/s, n=4).

Conclusion: This proof-of-concept study demonstrates that BioWire could re-establish cardiac conduction between isolated regions of two-dimensional cardiac tissue. The approach is highly generalizable, offering a novel platform to engineer biologically-compatible materials for relaying electrical signals.

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Molecularly Defined Re-Wiring of Electron Transport in Living Cells

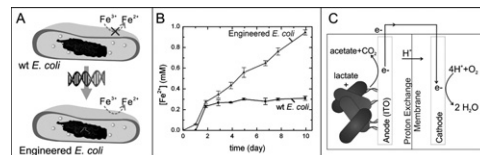
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Cellular-electrical connections have the potential to combine the specialties of the technological world with those of the living world. However, cell membranes are natural insulators, inherently creating a barrier between intracellular electrons and inorganic materials. To overcome this barrier, we have ‘grown’ electrical connections in living cells by engineering the cell to construct a well-defined electron conduit. The dissimilatory metal-reducing microbe, *Shewanella oneidensis* MR-1, inspired our approach: it has the unusual ability to transport electrons to extracellular minerals via a trans-membrane electron transport pathway (ETP). We seek to generalize this ability to grow electrical contacts between microbes and inorganic materials, and thus have genetically re-engineered a portion of the *Shewanella* ETP into *Escherichia coli* (Fig. A). Native *E. coli* proteins complete the partial ETP by acting as a direct electron donor to the functionally expressed *Shewanella* proteins. These ‘electrified’ strains exhibit ~8x and ~4x faster metal reducing efficiency with soluble metals and insoluble metal oxides, respectively, than wild-type *E. coli* (Fig. B). These experiments provide the first steps towards engineering of hybrid living-non-living systems.

Our next objective is to measure direct electrical output from the ‘electrified’ strains to an electrode (Fig. C).



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Sensitive and Selective Nucleic Acid Capture with Shielded Covalent Probes

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Understanding how the parts encoded in the genome interact to build and sustain life is one of the key challenges of modern biology, and nucleic acid probes are at the heart of many of the techniques used to probe these interactions. In vitro, they are used to identify genotypes and species, detect nucleic acid-protein interactions, and measure gene expression patterns in time and space. In vivo, synthetic nucleic acids are used to inhibit expression for research and therapeutic interventions. In each of these applications, however, the power of nucleic acid probes is limited by imperfect selectivity (binding of undesired targets) and incomplete affinity (not all desired targets are bound). These limitations stem from reliance on base pairing to both bind the desired target and discriminate against off-target sequences. Longer probes bind the designed target securely but can also pair imperfectly with undesired targets. Shorter probes or structured probes such as molecular beacons discriminate mismatches effectively, but at the cost of reduced affinity for the desired target.

We have recently developed a new technology, shielded covalent probes, in order to resolve the selectivity/affinity tradeoff. The probes are designed to fold into hairpins in the absence of target, ensuring selectivity through competition between probe-probe and probe-target pairing. Once hybridization is complete, photo-activatable crosslinkers form covalent bonds between the probe and target that are far stronger than the non-covalent interactions of base pairing. In vitro assays show that designed targets are covalently bound with nearly quantitative yield, and mismatches are efficiently rejected. Furthermore, the bonds are stable under extremely stringent conditions, enabling removal of uncross-linked molecules. The crosslinks can then be reversed to release the isolated target. We envision a wide array of applications for these probes.

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Discovery of VEGF-KDR Binding Inhibitors by Screening Small-Molecule Microarrays with Label-Free Ellipsometric Scanners

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Developing new small-molecule drugs against protein targets requires screening large collections of structurally diverse compounds for those with sufficiently high affinity to and inhibition effect on a protein target before further structural optimization and developmental work. Small-molecule microarrays (SMM) with a suitable binding assay platform are one of the viable high-throughput screening options. We demonstrate that by combining an oblique-incidence reflectivity difference optical scanner with microarrays, we can screen >10,000 compounds per glass slide for ligands to a protein target without fluorescence labeling. Using such a platform, we recently screened 8,000 small molecule compounds from the National Cancer Institute Developmental