ENGINEERING DNA-BASED NANOCANALLES AND VESICLES
FOR CONTROLLED MOLECULAR TRANSPORT

by

Yi Li

A dissertation submitted to Johns Hopkins University in conformity with the requirements for
the degree of Doctor of Philosophy

Baltimore, Maryland
April 2021
Abstract

For the past two decades, synthetic transmembrane nanopores and nanochannels have become powerful tools in biosensing and single-molecule studies. Due to the ease of rational design and advancements in DNA functionalization, DNA has been established to be versatile building blocks for the bottom-up fabrication of nanostructures. Recently, DNA-based nanopores in both small diameters (1-2 nm) showing transport of ions and small molecules and large diameters (5-10 nm) showing transport of proteins across lipid bilayer membranes were reported. Nevertheless, those DNA nanopores have lengths below 100 nm, and the molecular transport only occurs across lipid membranes. It remains unknown if longer nanochannels can be constructed for transport over extended distances. Such nanochannels of longer lengths can be potentially used as conduits for carrying molecules on the cell-size scale or between compartments apart.

We have designed a microns-long DNA nanochannel 7 nm inner diameter that inserts onto the lipid membranes of giant unilamellar vesicles and allows the transport of small molecules through its barrel. Kinetics analysis suggests a continuum diffusion model can describe the transport phenomenon within the DNA nanochannel. The reduced transport upon bindings of DNA origami caps to the channel ends reveals the molecules mainly transport from one channel end to the other rather than leak across channel walls. We further design a DNA nanopore-cap system that responds to specific DNA sequences. In combination with giant unilamellar vesicles that encapsulate glucose molecules, we present a biosensor system consisting of capped DNA nanopores and vesicles that can detect and amplify nanomolar DNA signals millimolar glucose outputs. The DNA-based biosensor we developed shows the potentials to be used as point-of-care nucleic acid diagnostic devices.
Another challenge in using DNA nanochannels or other DNA-based nanostructures in biological environments or cell culture is that they may be degraded by enzymes found in these environments, such as nucleases. To improve the DNA nanostructures' stability, we demonstrate a means by which degradation can be reversed in situ through the repair of nanostructure defects. The ability to repair nanostructures, such as DNA nanochannels, could allow particular structures or devices to operate for long periods of time and might offer a single means to resist different types of chemical degradation.

**Thesis Advisor:**
Professor Rebecca Schulman
Department of Chemical & Biomolecular Engineering and Department of Computer Science

**Thesis Committee Members:**

**Professor Jeffrey Gray**
Department of Chemical & Biomolecular Engineering

**Professor Michael Tsapatsis**
Department of Chemical & Biomolecular Engineering

**Professor Kalina Hristova**
Department of Materials Science and Engineering and Department of Biomedical Engineering

**Professor Taekjip Ha**
Department of Biophysics and Biophysical Chemistry
Acknowledgment

First and foremost, I am extremely grateful to my advisor Prof. Rebecca Schulman, who has led me into the group and guiding me through the methodology of scientific research. Prof. Schulman has always been particularly patient, caring, and supportive during my journey of scientific discoveries at Hopkins. She never ran out of ingenious ideas which she could not wait to share with me, which encouraged me to also think critically and progressively. The resources, platform, and opportunities she enthusiastically provided established a solid ground where I could freely explore the wonders in science and engineering. I also want to thank her for supporting all my decisions in academics, research, and my career plans. I would not have completed this journey with enjoyments without her words of encouragements every time of meeting.

I wish to thank Dr. Dan Siegal at APL whom I collaborated with on research projects for his appreciation of my works and sharing some of the most brilliant visions that helped shape my works. I also would like to thank Dr. Sisi Jia for mentoring me as the beginning of my journey at Hopkins, helping me to get started with experimentation in the field of nanotechnology. She has then offered me continued support both as a mentor and as a close friend. I would like to extend my sincere thanks to Prof. Jeffrey Gray for whom I had pleasure working as a teaching assistant. Prof. Gray has served on my annual review committee and not only acknowledged my research progresses but also given me many important advices.

I must thank my boyfriend, Hao Liu, for his unrelenting support and love that helped me go through many difficult times. I will always remember the very many wonderful moments we have spent together. Finally, I would never forget my friends at Hopkins — Qi Huang, Yuqi
Zhang, Runchen Zhao, Jianli Zhang, Hong Zhang, Yi Li, Tian Zhu, Danyu Wang, Jiawei Ge, and Kuan-Lin Chen, and Heon Joon Lee. They had brought so much happiness into my studies and my life for the past five years at Hopkins, and I believe the friendship is for a lifetime. I would not have got here without their caring, help, and encouragements.
Dedication

This thesis is dedicated to my parents, Xiaoyi Li and Wenxia Zhang, for their eternal love.
Table of Contents

Abstract .................................................................................................................................. iii

Acknowledgment .................................................................................................................... v

Dedication .............................................................................................................................. vii

Table of Contents .................................................................................................................. viii

List of Tables ............................................................................................................................ x

List of Figure ............................................................................................................................ xi

List of Supplementary Figures ............................................................................................ xiii

Chapter 1: Introduction ........................................................................................................... 1

1.1 Biological and Synthetic Membrane Nanopores ................................................................. 1

1.2 DNA-based Nanopores .......................................................................................................... 3

1.3 Nanopore Biosensors .......................................................................................................... 5

1.4 DNA Nanostructure Stability ............................................................................................. 8

1.5 Previous Work on DNA Nanotubes .................................................................................. 10

1.6 Dissertation Overview ....................................................................................................... 13

Chapter 2: Design DNA Origami Nanopores and Self-assembled Nanochannels ................. 14

2.1 Introduction ....................................................................................................................... 14

2.2 Methods ............................................................................................................................ 15

2.3 Results and Discussion ..................................................................................................... 18

2.4 Supplementary Information ............................................................................................ 21

Chapter 3: Characterize Molecular Transport through DNA Nanopore and Nanochannel with Giant Unilamellar Vesicles ......................................................................................... 40

3.1 Introduction ....................................................................................................................... 40

3.2 Methods ............................................................................................................................ 44
List of Tables

Table S2.1. Names for the 72 staple strands of the DNA nanopore. ................................. 22
Table S2.2. Sequences of the 12 nanopore staples for cholesterol modified strand attachment. 22
Table S2.3. DNA cap staple sequences. ........................................................................................ 33
Table S3.4. Kinetic parameters for vesicles in Figure S3.2. .......................................................... 70
Table S3.5. Kinetic parameters for vesicles in Figure S3.4. .......................................................... 73
List of Figure

Figure 1.1. The four classes of pores featuring the strengths and challenges of the building materials. ................................................................................................................................. 2
Figure 1.2. DNA origami nanopores. ..................................................................................................................... 5
Figure 1.3. Electrophysiological measurements of transport through nanopores. ............................................. 7
Figure 1.4. DNA origami nanostructures denatures in physiological cation concentrations and are digested by nuclease enzymes present in fetal bovine serum (FBS) used as a medium supplement. ................................................................................................... 9
Figure 1.5. Schematic of the design of DNA nanotubes, seeds, and the growth process. ............................ 11
Figure 1.6. Schematic of a protein-oligonucleotide signal exchange process. .............................................. 12
Figure 2.1. A DNA origami nanostructure functionalized on one end with hydrophobic moieties serves as a trans-membrane pore .............................................................................. 18
Figure 2.2. TEM characterization of DNA pores and DNA nanotubes with small unilamellar vesicles (SUVs). ........................................................................................................................................ 20
Figure 2.3. DNA cap and capped nanochannel ................................................................................................. 21
Figure 3.1. Scheme for studying end-to-end transport of small molecules through micron-length self-assembled DNA nanochannels ............................................................................ 43
Figure 3.2. TAMRA transport through open and capped DNA origami nanopores ..................................... 48
Figure 3.3. Rates of dye diffusion through DNA nanotube channels into GUVs suggest dye diffuses end-to-end through nanotube channels ........................................................................ 54
Figure 4.1. Capping and uncapping of DNA nanopores .................................................................................. 54
Figure 4.2. Encapsulation and release of glucose molecules from GUVs .......................................................... 96
Figure 4.3. Capped DNA nanopores and GUVs encapsulating glucose can be used for fast detection of target DNA species .............................................................................................. 99
Figure 4.4. Plot of DNA key concentration responses to different initial concentrations of thrombin for 50 nM DNA aptamer and transduction strands .............................................. 102
Figure 5.1. DNA nanotubes self-assemble from DNA tiles via hybridization of complementary single-stranded DNA sticky ends ............................................................................ 113
Figure 5.2. Kinetic measurements of nanotube self-healing and degradation in serum. .......................... 115
Figure 5.3. Incorporation of DNA Tiles into Nanotubes .................................................................................. 122
Figure 5.4. Simulated nanotube degradation and repair and differences in the dynamics of nanotube degradation and nanotube coupled degradation and repair observed in experiments. ............................................................................................................ 124
List of Supplementary Figures

Figure S2.1. Schematic showing the architecture of the DNA tiles. ............................................................... 24
Figure S2.2. Schematic of the monomer activation reaction. ........................................................................... 25
Figure S2.3. A coarse-grained model of DNA nanopore .................................................................................. 29
Figure S2.4. Example fluorescence micrographs of DNA nanotube channels attached to DNA pores. ...................................................................................................................... 30
Figure S2.5. Additional TEM Images of DNA origami nanopores without added DNA-cholesterol conjugates. .......................................................................................................................... 32
Figure S2.6. Additional TEM Images of DNA nanopores interacting with SUVs .................................................. 32
Figure S2.7. Design of the DNA channel cap .................................................................................................... 33
Figure S2.8. A structural snapshot from a coarse-grained model of the DNA cap. ........................................ 34
Figure S2.9. Additional TEM Images of DNA origami caps ............................................................................ 35
Figure S2.10. Example wide-field fluorescence image of DNA caps bound to DNA nanopores. .................................................................................................................................................... 36
Figure S2.11. Design of the DNA seed pore .................................................................................................... 37
Figure S2.12. Simulated structure of the seed pore ............................................................................................ 38
Figure S2.13. TEM image of DNA seed pores assembled in TAEM buffer ....................................................... 39
Figure S3.1. Example fluorescence micrographs of GUVs from the dye influx experiment with DNA nanopores. ........................................................................................................................................ 65
Figure S3.2. Example plots of measured fractional intensities of GUVs and corresponding regression curves. .............................................................................................................................................. 68
Figure S3.3. The probability density distributions of the influx rates in the nanopore and capped nanopore dye influx experiments. ........................................................................................................ 71
Figure S3.4. Example plots of measured and fitted fractional intensities of GUVs in experiments where DNA nanochannels are added to GUVs ...................................................................................... 72
Figure S3.5. Fourier transform of the probability density distribution of influx rates of TAMRA into DNA seed pores. ......................................................................................................................... 76
Figure S3.6. Schematic of the dye influx experiment setup .................................................................................. 76
Figure S3.7. Bright field microscopy image of GUVs formed from POPC lipids .................................................... 77
Figure S3.8. Confocal fluorescence image of GUV formed using gel-assisted hydration method. ........................................................................................................................................................................ 78
Figure S3.9. TEM image of DNA nanopores that were incubated in TAE buffer supplemented with 500 mM potassium chloride for an hour at room temperature ......................... 79

Figure S3.10. Bright field image of GUVs in 3 mM magnesium acetate ....................................... 80

Figure S3.11. TEM image of DNA nanopores that were incubated in TAE buffer supplemented with 3 mM magnesium acetate .............................................................................. 81

Figure S3.12. Confocal fluorescence image of PEG-coating nanochannels and GUVs .............. 82

Figure S3.13. Fluorescence images of the same volumes of DNA nanochannels before dialysis and after dialysis .................................................................................................... 83

Figure S4.1. Fluorescence calibration curve of FAM fluorophore ........................................... 104

Figure S5.1. Multicolor fluorescence images showing seeded nanotubes without PEG coating .......................................................................................................................... 130

Figure S5.2. Primary amine-modified SEs tiles are conjugated to succinimidyl valeric acid PEG with a molecular weight of 20 kDa (PEG20k-SVA) ........................................................................ 131

Figure S5.3. Schematic of the reaction in which the activation strand reacts with an inactive tile by displacing the strand that covers one of the sticky ends......................................... 132

Figure S5.4. Schematic of the assembled adapter tiles for the seeds .................................... 134

Figure S5.5. Schematic illustrations of the passivated glass surface and the anchoring of DNA nanotube seeds to the surface via biotin-NeutrAvidin chemistry ................................................ 139

Figure S5.6. Schematic illustration showing a biotin attachment linker strand on a seed............ 141

Figure S5.7. PEG-coated seeded nanotubes ................................................................................ 142

Figure S5.8. Sample AFM images of PEG coated DNA nanotubes ........................................ 143

Figure S5.9. Additional fluorescence microscopy images showing the breakdown of end-anchored PEG-coated seeded nanotubes after different incubation times in four different conditions ........................................................................................................ 144

Figure S5.10. Additional multicolor time-lapse fluorescence microscopy images of tile incorporation .................................................................................................................. 145

Figure S5.11. Simulated degradation and repair of nanotubes .............................................. 146

Figure S5.12. Fluorescence images of nanotubes grown in solution to which free tiles with different fluorescence labels are added .......................................................... 149

Figure S5.13. Monochrome fluorescence images of tile activation ...................................... 150

Figure S5.14. Schematic showing the architecture of U tiles ................................................ 151
Figure S5.15. Fluorescence images of U tiles and multicolor images of seeded nanotubes on dish glass surface. ........................................................................................................................................... 154

Figure S5.16. Time-lapse fluorescence images showing degradation of seeded DNA nanotubes. ........................................................................................................................................... 155

Figure S5.17. PAGE gel-electrophoresis image of the DNA-PEG=20kDa conjugation............ 155

Figure S5.18. Multicolor fluorescence image of nanotubes (Cy3, yellow) assembled on seeds (red, Atto647) with a PEG-40kDa coating. ......................................................................................... 157

Figure S5.19. Fluorescence images of nanotubes with a PEG-40kDa attached onto a glass- bottom dish. ........................................................................................................................................... 157
Chapter 1: Introduction

1.1 Biological and Synthetic Membrane Nanopores

Compartmentalization is one of the critical characteristics of living systems. Compartments separated by membrane barriers improve biological process efficiencies by concentrating reactants in confined space and protecting biological components from the external environment.\(^1,2\) To enable the communications and component distributions among the components, organisms evolved different transporters as the fundamental mechanisms for directing transport across membranes in living systems. The membrane transporters in nature are proteins, and each protein transports a specific class of molecule, such as ions, sugars, and amino acids.\(^3-6\) These proteins play a vital role in living organisms regulating ion/molecule transportation in retinal, neural, and muscular functions.\(^7\) Membrane proteins can be categorized into two major classes, carrier proteins and channel proteins. Carrier proteins bind to substances, which triggers a conformational change of the protein to move the substance across the membrane. Channel proteins form aqueous pores across the membrane that only weakly interact with the substances and switch between the gated state and open state.\(^3\) The membrane proteins are also often classified into nanopores and nanochannels. A nanopore is a small hole in the membrane with a length comparable to the pore diameter, and a nanochannel is the one with a length much larger than the radius.\(^8,9\)

Using protein nanopores in sensing applications has received much attention in the past two decades due to their high sensitivity and versatility.\(^10\) Compared to other sensing technologies, the nanopore has advantages by offering single-molecule detection and real-time
recognition with minimal sample volumes. The biological protein pores have been reported to detect a wide range of analytes, including metal ions, small molecules, nucleotides, and proteins. There are also tremendous interests in engineering protein nanopores for DNA/RNA sequencing, which could become the key technology in developing third-generation sequencing. However, the engineering or de novo design of protein pores is still a challenging task due to the lack of generic design rules. Thus, researchers are seeking alternative approaches for developing nanopores with more straightforward design principles and better tunability.

![Figure 1.1](image)

Figure 1.1. The four classes of pores featuring the strengths and challenges of the building materials. Image from Reference; protein, Ref. AAAS; peptide, Ref.; DNA, Ref. Macmillan Publishers Ltd; synthetic organic, Ref. American Chemical Society.

Taking inspiration from biological membrane proteins, artificial nanopores and nanochannels that mimic the functions of protein channels can be constructed. In the past several years, the advances in the synthetic nanopores not only improve the understandings of natural protein transporters but also have provided powerful tools for detecting specific biomolecules and studying single-molecule biophysics.
Synthetic membrane nanopores can be made from different materials, including peptides, organic polymers, DNA, carbon nanotubes, and more.\textsuperscript{10} Synthetic nanopores tend to be more tunable in size, geometry, and surface chemistry. However, it is challenging to construct nanopores with the same degree of specificity and biochemical selectivity as the protein pores.\textsuperscript{36} To construct nanopores that can fully mimic the functions of membrane proteins found in nature, it is essential to investigate the transport phenomena within the nanoscale confinements of various synthetic nanopores.\textsuperscript{37,38} Studies have shown that transport phenomena within the nanopores and nanochannels, where interfacial effects at channel walls became important, could differ from the continuum descriptions.\textsuperscript{36} Notably, the physical properties of solutes, chemical factors of wettability, charge, and host-guest recognition can affect the transport within the channels.\textsuperscript{39–41}

1.2 DNA-based Nanopores

DNA nanotechnology has established DNA to be versatile building blocks for the bottom-up fabrication of nanostructures.\textsuperscript{42,43} Taking advantage of the programmability of Watson-Crick base pairing, DNA can be rationally designed to form complex structures with nanometer precisions in geometry. The advancement in DNA functionalization chemistry allows DNA nanostructures to interface or reorganizing the arrangement of other molecules.\textsuperscript{40,42,44} Among different DNA self-assembly techniques, the DNA origami technique is easy to use and flexible enough to be used to construct a variety of 2D and 3D structures.\textsuperscript{45} DNA origami relies on the process in which long single-stranded scaffold plasmid strand hybridizes with many short staples strands during thermal annealing to fold into the target structure. This process is generally
reliable, generating a high yield of the target structure. Computer software has further streamlined the DNA origami designs.46–53

Taking advantage of the vast design space of DNA nanostructures, researchers developed a variety of transmembrane DNA-based nanopores.19 These DNA nanopores have different pore heights and wall thicknesses but mostly consist of parallel-aligned DNA duplexes with a stem domain penetrating the membrane.54,55 Due to the negative charge of DNA, the electrostatic interaction between DNA and lipid membranes strongly depends on the lipids' charges. DNA is electrostatically attracted to polar or positively charged lipids, particularly at low salt concentrations. DNA can also bind to the zwitterionic lipid layers in the presence of divalent cations, likely due to the divalent cations inserting the lipid molecules and bridging lipid headgroups and DNA backbone.49,53,56 Although DNA can bind to lipid membranes electrostatically, the energetic cost for inserting DNA into the hydrophobic core of a lipid bilayer is prohibitively high. However, this hole-formation energy barrier may be overcome by decorating DNA structures with hydrophobic moieties, such as cholesterol48, porphyrin50, tocopherol47, or ethyl group48.

Many DNA channels consist of several DNA strands, especially the 6-helix bundle (6HB) design, which has an inner diameter of about 2 nm. The open or close functions of 6HB nanopores have been demonstrated through voltage-gating26 and DNA ligand binding57. Such ability to regulate the target translocation can be potentially used in drug delivery and synthetic cells.50–53 However, due to the small channel size, the studies of 6HB nanopores have focused on ionic current measurements. Recently, translocation of larger molecules has been demonstrated with large DNA nanopores that have inner diameters of 5–10 nm.50–53 These large-diameter
structures gained interest for their extended range of analytes for point-of-care diagnostics and environmental screening.

**Figure 1.2.** DNA origami nanopores. (A) Schematic illustration and TEM images of the transmembrane channel. Ref.49 AAAS. (B) Design and AFM images of DNA origami nanopores. Ref.53 ACS Publications. (C) Design of the T-shape pore, composed of a double-layered top plate (gray) and a 27 nm-long stem (red). Ref.58 Springer Nature. (D) Synthetic protein conductive membrane DNA nanopore. Ref.52 Springer Nature. Image from Reference.57

**1.3 Nanopore Biosensors**

Biosensors developed through the engineering of biological processes, including enzyme reactions, antibody and DNA bindings, and cell organelles, have become an attractive field of study.59–61 They serve as analytical devices that convert biological responses into signals that can be read by electronic devices. Many recent implementations are shown to address the unmet
needs in medical diagnostics, drug discovery, food safety, and environmental monitoring. In medical science, biosensors have enabled early-stage detections of heart diseases and the human papillomavirus.

Biosensors must meet the requirements of high specificity and sensitivity for the target biological signals. The recent development in nanotechnology has vastly increased the sensitivity limit of biosensors.\textsuperscript{60,62} Nanostructures have unique properties of large surface area to volume ratio and mass transfer rates, significantly enhancing the biochemical bindings and signal to noise ratio. The advancements in the surface chemistry of nanostructures and nanoparticles have expanded the design space for tailoring the surfaces for target recognitions.\textsuperscript{63}

Among various forms of nanostructures, nanopores emerged to become a powerful tool for detecting, analyzing, and sensing single molecules.\textsuperscript{64} Nanopores that detect a broad range of analytes, including proteins, peptides, drugs, polymers, ions, nucleotides, and other macromolecules, have been reported. The nanopore-bases sensing is generally label-free and amplification-free. By monitoring the conductivity or ion trafficking of the pores embedded in a lipid membrane, translocation events of the target ions or molecules can be quantitatively detected as a characteristic blockade current and duration.\textsuperscript{65} Thus, the nanopores-based sensors can detect analytes at single-molecule resolution without the needs for labels or tags. Several well-characterized protein pores are widely used for biosensing applications, such as α-hemolysin, Mycobacterium smegmatis porin A, alamethicin and phi29.\textsuperscript{66}
Although protein pores have been shown to detect various analytes reliably, they also exhibit disadvantages such as fixed pore size and limited stability.\textsuperscript{70} Many synthetic nanopores have been developed to explore a much more extensive range of pore geometries. Solid-state nanopores, i.e., nanopores fabricated from solid-state materials, present advantages of high stability and adjustable sizes and surface properties. Recently, with advances in ion beam sculpting technique, solid-state nanopores can be fabricated with sub-nanometer control over pore diameters, further expanded the range of potential applications.\textsuperscript{70–72} By functionalizing the nanopores' surface with a large number of specific recognition sequences or receptor molecules,
the translocation of the molecules with desired biochemical properties can be detected through ionic current measurements.

Besides the solid-state nanopores, membrane-embedded nanopores consisted of peptides, DNA, or polymers are used in biosensing.\textsuperscript{19,73} Peptide pores are usually smaller than protein pores and can be built from non-biogenic amino acids through solid-phase synthesis, offering vast chemical parameter space.\textsuperscript{73} Two examples of polymer-based nanopores are stacks of macrocycles\textsuperscript{74} and carbon nanotubes\textsuperscript{75}. Both types of nanopores have shown positive detection of both ion transport and DNA translocation through ionic current measurements.

Benefited from the programmability of DNA interactions, DNA has been used to construct nanopores with different pore heights and diameters. Recently, DNA origami nanopores that can switch to an open or closed state upon specifically capturing target molecules at pre-designed sites have been reported.\textsuperscript{26,51} The target molecules can be specific DNA sequences, captured via DNA hybridizations, or proteins that are recognized through aptamer bindings. Since the molecular transport through the nanopores is controlled by the opening and closing of the nanopores, the recognition of the target molecules can be reported as changes in molecular fluxes across the membranes in which nanopores are inserted.

1.4 DNA Nanostructure Stability

As DNA nanostructures, including DNA nanopores, have become powerful tools in diagnostics and therapeutics for their spatial addressability, mechanical flexibility and biocompatibility.\textsuperscript{76–79} One of the main challenges to the use of DNA nanostructures in drug delivery and for building biosensors is their rapid degradation in cell culture or in vivo by
nucleases, which may be released as dying cells burst or be secreted.\textsuperscript{80,81} Fetal bovine serum (FBS), which contains various nuclease enzymes, at 37 °C is commonly used as a model system for understanding how DNA nanostructures might function in in vitro cell culture or in vivo and for characterizing their degradation. Typically, unmodified DNA nanostructures are completely degraded within 24 h when incubated in 10% FBS.\textsuperscript{82–85}

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{figure1.png}
\caption{DNA origami nanostructures denatures in physiological cation concentrations and are digested by nuclease enzymes present in fetal bovine serum (FBS) used as a medium supplement. Image from Reference.\textsuperscript{80} ACS Publications.}
\end{figure}

A variety of approaches have been reported to enhance stability of DNA nanostructures in serum. However, those reported strategies focused on protecting DNA nanostructures to improve their stability in biological environments. Chemical modifications or coatings of either DNA strands or nanostructures must be devised specifically to achieve this protection, which for some modifications can be labor- or cost-intensive. Modifications to DNA nanostructures, such as the conjugation of DNA to other charged molecules,\textsuperscript{86,87} could also compromise the biocompatibility of DNA materials which would be undesirable for some applications.
1.5 Previous Work on DNA Nanotubes

1.5.1 Self-assembly of DNA nanotubes

In 2004, Paul Rothemund et al. presented a bottom-up approach for constructing nanotube structures using DNA as the building material. DAE-E DNA tiles, consisting of five DNA oligomers with two crossovers presenting four single-stranded DNA sticky ends, are formed during a typical annealing process. The sticky ends on the DNA tiles can be programmed to hybridize to other tiles to join onto each other to form a lattice during an isothermal incubation. The lattice then cyclizes to form nanotubes due to the inherent curvatures of the tiles.

To gain spatial and temporal control over DNA nanotube assembly, Abdul Mohammed and Rebecca Schulman developed a nucleation pathway for nanotube formation. Because at low supersaturation, tile joining by 2 two sticky ends is more favorable than joining by one sticky end, a stable nucleus structure from which nanotubes can grow would reduce the energy barrier to nucleation. Thus, nanotubes would preferably grow from a preformed seed, which serves as a stable nucleus, rather than nucleating homogeneously without seeds (Fig. 1.5). In this study, the seed is designed to present a template for nanotube circumference so that the nanotubes formed in the seeds' presence have the same circumference as the seed. The seed is built using the DNA origami technique and consists of 12 parallel DNA helices connected by crossovers between helices. The hairpins on the seed are used to differentiate between the inner surface and outer surface by preventing the seed from cyclizing with hairpins inside the structure. The study further shows the presence of seeds increases the number of nanotubes that grow and total nanotube length. Simulations of DNA nanotube nucleation and growth can accurately predict the nanotube number and lengths, suggesting the self-assembly process can be quantitatively controlled.
The DNA nanotubes self-assembled from DNA tiles were later showed to grow to connect pairs of molecular landmarks. Such connections are stable and can span a surface or three dimensions. Using DNA capping structures, called termini, are then used to organize nanotubes into larger architectures. Moreover, the selective regulation of the termini binding affinity for DNA tiles can lead to the reconfiguration of these architectures. DNA nanotubes can also be used as templates to fabricate gold nanowires, to be potentially used in nanoscale electronics and sensing.

Figure 1.5. Schematic of the design of DNA nanotubes, seeds, and the growth process. At low supersaturation, seeded nucleation is much faster than unseeded nucleation. Image from Reference.89

1.5.2 Modular thrombin-binding aptamer system

Human α-thrombin is a multifunctional serine protease and is essential in thrombosis, homeostasis, and inflammation. Thus, thrombin-binding aptamers are of particular interest in medical and clinical field. Agrawal and Schulman developed a reaction process that transduced
the thrombin protein signal to an oligonucleotide signal. A thrombin-binding DNA aptamer that interacted with thrombin proteins reported the thrombin concentration as the concentration of an output DNA oligonucleotide strand. Specifically, the aptamer system consists of two stages. The first stage is for thrombin recognition, in which the presence of thrombin would lead to reversible aptamer-thrombin binding. The second stage is to transduce input signals to DNA outputs using reversible toehold-mediated strand displacement reactions. A change in the concentration of free aptamers would lead to a change in the DNA output strand. The nucleotide sequence of the output strand is almost independent of the sensing protein, and therefore this system can be easily modulated to produce a DNA output strand with the desired sequence, which can then participate in any downstream reactions.

Figure 1.6. Schematic of a protein-oligonucleotide signal exchange process. Protein concentration is reported as the concentration of an output oligonucleotide with the designed sequence. The concentration of the output strand (O1) depends on the concentration of the input protein (P1) and the initial
concentrations of the sensor components (W1, X1Y1, O1Z1). The aptamer (W1) binds to the input protein (thrombin for Sensor W1-O1) to form an aptamer–protein (W1P1) complex. Aptamer strands that are not bound to the protein can react with the X1Y1 complex, shifting the equilibrium of a reversible reaction so as to change the concentration of free O1. Image from Reference^95.

1.6 Dissertation Overview

This thesis consists of three aims. 1) Construct DNA-based nanopores and nanochannels for transporting small molecules across membranes and for longer distances. 2) Use DNA nanopores and vesicles to build biosensor devices for nucleic acid detection. 3) Develop a strategy for extending DNA nanostructure lifetime in biological conditions.

Specifically, I first show the formation of nanopores using DNA origami technique and nanochannels are self-assembled from DNA tiles. Both DNA nanopores and nanochannels can spontaneously insert onto lipid bilayer membranes after hydrophobic modifications (chapter 2). Protocols for preparing giant unilamellar vesicles (GUVs), a commonly used model system for cellular membranes, in different conditions are developed. I then investigate the transport mechanism and transport rates of a small molecule dye through DNA nanopores and nanochannels through quantitative studies using GUVs (chapter 3). The nucleic acid biosensor application of DNA nanopores is explored (chapter 4). Lastly, I have developed a self-healing mechanism for maintaining the DNA nanostructures for longer period of time in serum (chapter 5). I then conclude the thesis and discuss the future directions for the research described in the thesis (chapter 6).
Chapter 2: Design DNA Origami Nanopores and Self-assembled Nanochannels

2.1 Introduction

Nanoscale channels are fundamental mechanisms for directing transport across membranes in living systems. Synthetic nanopores and nanochannels that mimic biological transporters' functions have become powerful tools for detecting specific biomolecules and studying single-molecule biophysics.8,10,18,19,24,25 Biomimetic nanochannels have been constructed to explore molecular transport within nanoscale confinement using proteins, silicon, polymer, and carbon nanotubes.9,25,36,96 Studies showed that interfacial effects at channel walls could play an essential role in the ion and molecule transport within nanochannels, leading to a transport phenomenon very different from the continuum descriptions.36,37,97,98

Unlike the protein channels are typically 1-2 nm wide, transmembrane nanopores and nanochannels built with synthetic materials have overcome the size limitations in the past few years. Synthetic nanopores of 5-10 nm inner diameters were built, which allowed diffusive transport of various small molecules and folded proteins.29,50–52,99 These large-diameter structures gained interest for their extended range of analytes for point-of-care diagnostics and environmental screening. However, the behaviors of molecular transport within these large nanochannels were less studied. Whether the surface effects played an important role in molecular transport is still not well understood.

The advancements in DNA nanotechnology have enabled the construction of DNA nanostructures with nanometer-precision control over the geometries. The DNA origami technique is widely used to build DNA nanopores of different sizes. In 2012, Langecker et al.
reported an assembled stem structure of 2-nm inner diameter using DNA origami. To overcome the energy barrier for inserting the negatively charged DNA structures into the lipid bilayers, the nanopore was labeled with cholesterol molecules to interact with the hydrophobic core of lipid bilayer membranes. This synthetic DNA nanopore showed translocations of single-stranded DNA molecules. Later, large-diameter DNA-based nanopores were recently reported. These large-diameter DNA nanopores demonstrated transport of ions, small molecules, nucleic acids, and folded proteins. Nevertheless, existing DNA nanopores act as cross-membrane transporters with lengths not exceeding 100 nm. It remains unknown whether DNA-based nanochannels in both large diameter and longer lengths for the end-to-end passage of molecules can be constructed. If so, such nanochannels can be used as conduits that enable bulk transport of a wide range of different molecules over longer distances.

Here, we constructed DNA nanochannels of 7-nm inner diameter and lengths up to several microns through self-assembly. We started by designing and synthesizing a DNA nanopore using the DNA origami technique. The nanopore could spontaneously insert onto lipid membranes after being modified with twelve cholesterol molecules. We then showed DNA nanochannels of the same diameter and micron lengths were self-assembled on the DNA origami nanopores from DAE-E double-crossover DNA tiles also inserted onto lipid membranes after cholesterol modification. The mechanisms and rates of small molecule transport within the DNA nanopores and nanochannels are discussed in chapter 4.

2.2 Methods

Materials
All DNA oligodeoxynucleotides were purchased from Integrated DNA Technologies (IDT, Coralville, Iowa, USA), except the 7,240bp M13mp18 scaffold strand purchased from Bayou Biolabs (Los Angeles, CA, USA). Tris/acetate/EDTA (TAE) buffer was purchased from ThermoFisher Scientific (Waltham, MA, USA). 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) was purchased from Avanti Polar Lipids (Alabaster, Alabama, USA).

**DNA nanopore assembly**

A mixture containing 5 nM scaffold strand, 200 nM staple strand mix, and 25 nM attachment strand mix (Supplementary 2.4.1), was first prepared in TAE Mg2+ buffer (40 mM Tris-Acetate, 1 mM EDTA) with 12.5 mM magnesium acetate added (TAEM) (Supplementary Note 2.4.3). The assembly mixture was subjected to a thermal annealing ramp with an Eppendorf Mastercycler (Supplementary Note 2.4.3). The assembled nanopores were then purified to removed excessive DNA strands using a 100k MWCO Amicon Ultra centrifugal filter device (Millipore). Following purification, 0.15 μl of 100μM ATTO647-modified DNA strand that hybridizes to binding sites on attachment strands was added to 40 μl purified seed pore sample and incubated at room temperature for 15 min to allow fluorescently label the seed pores. The concentration of fluorescently labeled pores was approximately 1 nM, determined by measuring the concentration of a stock solution by counting the number of seed pores per field of view (86 μm × 86 μm) adsorbed to a glass slide from a specific reaction volume in fluorescence micrographs captured using a fluorescence microscope (Olympus IX71) with a 60×/1.45 NA oil immersion objective lens and a 1.6x magnifier lens. To modify the DNA nanopores with cholesterol, 1 μl DNA-cholesterol conjugate (Supplementary 2.4.3) at 10 μM was incubated at
50 °C for 10 minutes to alleviate cholesterol aggregation before being added to 40 μl purified DNA nanopores. The solution was then incubated for 10 minutes at room temperature.

**DNA nanotube channel assembly**

DNA nanotube channels were self-assembled from DNA tiles and DNA nanopores. DNA tile monomers in an inactive form and DNA nanopores with adapters that presented monomer sticky ends were separately prepared before being mixed to form nanotube channels. Inactive DNA monomers were prepared by mixing six strands that fold into inactive monomers (Supplementary 2.4.2), each at 400 nM in TAEM and subjecting the mixture to a thermal annealing ramp (Supplementary 2.4.3). To make DNA nanopores with adapters, the seed assembly mixture was prepared as in the “DNA pore assembly” section except that 100 nM adapter strands were added to the mixture before thermal annealing (Supplementary 2.4.3). After assembly, the nanopores were purified and concentrated to 2 nM, then fluorescently labeled. 20 μl of seed pores were then mixed with 20 μl inactive monomers and 0.2 μl monomer activation strand (50 μM). The sample was then incubated at 37 °C for at least 15 hours to allow the nanotubes to grow. DNA-cholesterol conjugates were then hybridized to the pore regions of the nanotube channels by following the same steps used for modifying nanopores before influx assays.

**SUV Preparation**

5 mg 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC, Avanti Polar Lipids, USA) was dissolved in 1 ml chloroform in a glass vial. The solvent was evaporated by blowing nitrogen gas into the glass vial for 20 minutes so that a dry lipid film was formed. The glass vial
was kept in a vacuum desiccator overnight to remove any residual solvent. 1 ml of 150 mM potassium chloride solution was then added to the glass vial to hydrate the lipid film. SUVs were then formed by 2-hour sonication in a bath sonicator (Branson Ultrasonic Cleaner 1510R-DTH) at 40 °C. The SUVs were diluted 1,000 times with 150 mM potassium chloride before use.

2.3 Results and Discussion

We first designed the DNA nanopore by modifying a 12-helix scaffolded DNA origami cylinder of length 63.1 ± 1.8 nm, a nanotube seed, that could act as a template for DNA nanotube growth (Fig. 2.1 left). We added 15-nt extensions to 12 of the staple strands near one end of the structure to which 12 cholesterol conjugated single-stranded DNA strands could hybridize. Coarse grained simulations predicted a pore diameter of 7 nm (Figure S2.3). The structure was formed by mixing M13 scaffold strand with 72 staple strands and 100 attachment strands for fluorescence labeling and performing a thermal annealing process (see Supplementary 2.4.1). The nanopore structure was then purified through filtration and modified with cholesterol by adding cholesterol-DNA conjugates to purified nanopores.

**Figure 2.1.** A DNA origami nanostructure functionalized on one end with hydrophobic moieties serves as a trans-membrane pore. DNA tiles hybridize to sticky ends on the DNA pore’s opposite end to self-
assemble a DNA nanotube hundreds of nanometers to microns in length that serves as a nanofluidic channel.

We imaged the nanopores and measured the length using transmission electron microscopy (TEM). Electron micrographs of >100 nanopores (Fig. S2.5) showed a mean length of 61.1 ± 0.5 nm, consistent with prior measurements via atomic force microscopy of the nanotube seeds. To verify that the cholesterol-modified nanopores could spontaneously insert into the lipid bilayer membranes, we synthesized small unilamellar vesicles (SUVs) and then mixed the cholesterol-modified nanopores with the SUVs. The electron micrographs showed the insertions of nanopores on the SUVs at orientations generally perpendicular to the membranes (Fig. 2.2a).
Figure 2.2. TEM characterization of DNA pores and DNA nanotubes with small unilamellar vesicles (SUVs). **a**, Schematic and images of cholesterol-modified DNA origami nanopores interacting with SUVs. **b**, Schematic and TEM images of cholesterol-modified DNA interacting with SUVs. Scale bars 20 nm (a), 100 nm (b).

Longer nanochannels by growing nanotubes were assembled from the DNA nanopores and were hundreds of nanometers to several microns in length (Fig. 2.4) during isothermal incubation at 37 °C (Fig. 2.1 right), and then attaching cholesterol-DNA conjugates to the nanopores. These channels could also insert into lipid bilayer membranes after being mixed with SUVs (Fig. 2d). These electron images also suggested that the inner diameter of the assembled DNA nanotubes was similar to that of the nanotube seed, which is consistent with the fact that both structures are assembled from the same number of helices in a similar crossover pattern.

We then asked the question whether the opening and closing of the DNA nanochannel can be controlled. To answer this question, we proposed to use a DNA nanostructure to plug the channel opening. Specifically, we designed a scaffolded DNA origami cap structure that can specifically bind to the open end of the nanochannel. The nanopore-cap binding was mediated by the complementary sticky ends presented on the adapters at the nanochannel open end and one end of the cap. To prevent small molecules to prevent small molecules from diffusing through the cap’s end, we cinched some of the cap’s staples to bring opposing helices together (Fig. 2.3a, Fig. S2.7). TEM images showed the caps were constricted, and the cap’s opening measured 1.1–1.4 nm in a coarse-grained simulation of DNA folding with oxDNA100 (Fig. S2.8). TEMs images showed caps were on average 60.4 ± 2.5 nm long (N=150) (Fig. S2.9), consistent with their designed length. To verify the DNA caps can bind to the nanopores, we mixed 2-fold excessive...
concentration of DNA caps, labeled with ATTO488 dye, with 0.3 nM DNA nanopores, labeled with ATTO647 dye and without cholesterol modification. Through fluorescence imaging, 97.3 ± 0.6% (SD, N=678) nanopores were bound to capped (Fig. S2.10).

Figure 2.3. DNA cap and capped nanochannel. a, Schematic of the DNA cap structure. b, TEM micrograph of the cap. c, Schematic of the DNA nanochannel bound to a cap.

2.4 Supplementary Information

2.4.1. DNA Nanopore Design

The sequences of the 12 staple strands (shown in red in Supplementary Table 1) to which cholesterol modified strands hybridize (“cholesterol strand”) are listed in Supplementary Table 2, and the sequences of the remaining 60 staple strands are those of the same names reported in Mohammed & Schulman\textsuperscript{90}. The 24 adapter strands, added along with staple strands at annealing, were the same as the A adapters reported in Jia et al\textsuperscript{101}.
Table S2.1. Names for the 72 staple strands of the DNA nanopore.

<table>
<thead>
<tr>
<th></th>
<th>T_5R2F_</th>
<th>T_5R2E_</th>
<th>T_3R_</th>
<th>T_3R_</th>
<th>T_1R_</th>
<th>T_1R_</th>
<th>T1R2_</th>
<th>T1R2_</th>
<th>T3R2_</th>
<th>T3R2_</th>
<th>T5R2_</th>
<th>T5R2_</th>
</tr>
</thead>
<tbody>
<tr>
<td>CholDN A.Tag1</td>
<td>CholDN A.Tag7</td>
<td>2F_H</td>
<td>2E_H</td>
<td>2F_H</td>
<td>2E_H</td>
<td>1F_H</td>
<td>1F_H</td>
<td>2F_HP</td>
<td>2E_HP</td>
<td>2F_HP</td>
<td>2E_HP</td>
<td>2F_HP</td>
</tr>
<tr>
<td>T_5R4_</td>
<td>T_5R4E_</td>
<td>T_3R_</td>
<td>T_3R_</td>
<td>T_1R_</td>
<td>T1R4_</td>
<td>T1R4_</td>
<td>T3R4_</td>
<td>T3R4_</td>
<td>T5R4_</td>
<td>T5R4_</td>
<td>T5R4_</td>
<td>T5R4_</td>
</tr>
<tr>
<td>CholDN A.Tag8</td>
<td>CholDN A.Tag9</td>
<td>1F_H</td>
<td>6F_H</td>
<td>1E_H</td>
<td>6E_H</td>
<td>1F_H</td>
<td>6E_H</td>
<td>2F_HP</td>
<td>2E_HP</td>
<td>2F_HP</td>
<td>2E_HP</td>
<td>2F_HP</td>
</tr>
<tr>
<td>T_5R6_</td>
<td>T_5R6E_</td>
<td>T_3R_</td>
<td>T_3R_</td>
<td>T_1R_</td>
<td>T1R6_</td>
<td>T1R6_</td>
<td>T3R6_</td>
<td>T3R6_</td>
<td>T5R6_</td>
<td>T5R6_</td>
<td>T5R6_</td>
<td>T5R6_</td>
</tr>
<tr>
<td>CholDN A.Tag9</td>
<td>CholDN A.Tag10</td>
<td>5F_H</td>
<td>8F_H</td>
<td>2E_H</td>
<td>8E_H</td>
<td>2F_HP</td>
<td>5F_HP</td>
<td>2E_HP</td>
<td>5F_HP</td>
<td>2E_HP</td>
<td>5F_HP</td>
<td>2E_HP</td>
</tr>
<tr>
<td>T_5R8_</td>
<td>T_5R8E_</td>
<td>T_3R_</td>
<td>T_3R_</td>
<td>T_1R_</td>
<td>T1R8_</td>
<td>T1R8_</td>
<td>T3R8_</td>
<td>T3R8_</td>
<td>T5R8_</td>
<td>T5R8_</td>
<td>T5R8_</td>
<td>T5R8_</td>
</tr>
<tr>
<td>CholDN A.Tag10</td>
<td>CholDN A.Tag11</td>
<td>8F_</td>
<td>12F_</td>
<td>10F_</td>
<td>12F_</td>
<td>8F_H</td>
<td>10F_H</td>
<td>10F_HP</td>
<td>8F_H</td>
<td>10F_HP</td>
<td>8F_H</td>
<td>10F_HP</td>
</tr>
<tr>
<td>T_5R10F_</td>
<td>T_5R10E_</td>
<td>T_3R_</td>
<td>T_3R_</td>
<td>T_1R_</td>
<td>T1R1_</td>
<td>T1R1_</td>
<td>T3R1_</td>
<td>T3R1_</td>
<td>T5R1_</td>
<td>T5R1_</td>
<td>T5R1_</td>
<td>T5R1_</td>
</tr>
<tr>
<td>CholDN A.Tag11</td>
<td>CholDN A.Tag12</td>
<td>12F_</td>
<td>12E_</td>
<td>12F_</td>
<td>12E_</td>
<td>12F_H</td>
<td>12E_H</td>
<td>12F_HP</td>
<td>12E_HP</td>
<td>12F_HP</td>
<td>12E_HP</td>
<td>12F_HP</td>
</tr>
<tr>
<td>T_5R12F_</td>
<td>T_5R12E_</td>
<td>T_3R_</td>
<td>T_3R_</td>
<td>T_1R_</td>
<td>T1R1_</td>
<td>T1R1_</td>
<td>T3R1_</td>
<td>T3R1_</td>
<td>T5R1_</td>
<td>T5R1_</td>
<td>T5R1_</td>
<td>T5R1_</td>
</tr>
<tr>
<td>CYC_C holDNA. Tag6</td>
<td>CYC_C holDNA. Tag7</td>
<td>12F_</td>
<td>12E_</td>
<td>12F_</td>
<td>12E_</td>
<td>12F_H</td>
<td>12E_H</td>
<td>12F_HP</td>
<td>12E_HP</td>
<td>12F_HP</td>
<td>12E_HP</td>
<td>12F_HP</td>
</tr>
<tr>
<td>T_5R12F_</td>
<td>T_5R12E_</td>
<td>T_3R_</td>
<td>T_3R_</td>
<td>T_1R_</td>
<td>T1R1_</td>
<td>T1R1_</td>
<td>T3R1_</td>
<td>T3R1_</td>
<td>T5R1_</td>
<td>T5R1_</td>
<td>T5R1_</td>
<td>T5R1_</td>
</tr>
<tr>
<td>CYC_C holDNA. Tag7</td>
<td>CYC_C holDNA. Tag8</td>
<td>12F_</td>
<td>12E_</td>
<td>12F_</td>
<td>12E_</td>
<td>12F_H</td>
<td>12E_H</td>
<td>12F_HP</td>
<td>12E_HP</td>
<td>12F_HP</td>
<td>12E_HP</td>
<td>12F_HP</td>
</tr>
</tbody>
</table>

Table S2.2. Sequences of the 12 nanopore staples for cholesterol modified strand attachment.

<table>
<thead>
<tr>
<th></th>
<th>TTGCAGCTACGTCATATGAGTTTTCAAAAGGAAACGTCCACCGTTTTTCGGTGGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_5R2F_CholDNA.Tag1</td>
<td>CTTA</td>
</tr>
<tr>
<td>T_5R4F_CholDNA.Tag2</td>
<td>TTGCAGCTACGTCTAAGGCTTTGCGTGCTTTGCCTTGGGACCACCTTG</td>
</tr>
<tr>
<td>T_5R6F_CholDNA.Tag3</td>
<td>TTGCAGCTACGTCTACCCGCTACAAAACGGCCGAACTCGGCCTTTTGGCGGA</td>
</tr>
<tr>
<td>T_5R8F_CholDNA.Tag4</td>
<td>CTG</td>
</tr>
</tbody>
</table>
The DNA nanopores were labeled with ATTO647 fluorophore dyes for fluorescence imaging. The labeling system consists of 100 attachment strands, each of which contains a subsequence that binds to the section of the M13mp18 scaffold that is not folded by staples. The remainder of each attachment strand binds to a labeling strand that has ATTO647 fluorophore dye on the 5’ end, “labeling_strand_ATTO647N”. The sequences of the attachment strands are the same as those listed in Mohammed and Schulman90.

cholesterol strand: TAGACGTAGCTGCAA/3CholTEG/
labeling_strand_ATTO647N: /5ATTO647NN/AAGCGTAGTCGGATCTC
/3CholTEG/ denotes a cholesterol molecule conjugated to the 3’ end of the DNA strand.
/5ATTO647NN/ denotes a ATTO647N (NHS Ester) fluorophore molecule conjugated to the 5’ end of the DNA strand.

2.4.2. DNA tile design
The DNA nanotubes are formed from the polymerization of oligomeric DNA monomers; nanotubes can either grow from or attach to DNA seed pores to form extended nanochannels with one end that can traverse a membrane. The tile design and sequence in this study are adapted from Rothemund et al. To make nanotubes that were stable at 37°C, we extended the sticky ends of the DNA tiles from 5 base pairs to 6 by shortening the double-stranded tile region so as to maintain a proper distance between crossover points. We began the study using nanotubes formed from DNA tiles as shown in Figure S1.

**Figure S2.1.** Schematic showing the architecture of the DNA tiles. Black triangles indicate crossover points. Cy3 fluorophores allow for nanotube visualization on the fluorescence microscope.

To ensure that monomers do not start to assemble into nanotubes before they are mixed with DNA seed pores, we designed modified monomers that could be assembled in an inactive form during annealing and could then be activated, i.e., reach a conformation that allowed assembly into nanotubes, by a strand-displacement reaction with an activation strand (Supplementary Figure 2). These monomers were based on those reported in Zhang et al. One of the sticky ends of the inactive monomers is double-stranded, which prevents the monomers from forming a lattice by sticky end joining. The activation strand, ‘SEs_activation’, upon addition to the solution, displaces the ‘SEs_inactive_strand5_right’ strand and exposes a single-
stranded sticky end where a double-stranded end was previously. The resulting products have four exposed sticky ends, allowing assembly of DNA nanotubes.

**Figure S2.2.** Schematic of the monomer activation reaction. The activation strand displaces the strand that covers one of the sticky ends to active the inactive monomer.

**SEs_1:** TCAGTGGACAGCCGTTCTGGAGCGTGACGAAACT

**SEs_2:** CCAGACAGTTTCGTGGTACCATCGTACCTC

**SEs_3-5’Cy3:** /CY3/CCAGAACGGCTGTGGCTAAACAGTAACCGAAGCACCAACGCT

**SEs_4:** GTCTGGTAGAGCACCACTGAGAGGTA

**SEs_inactive_strand5_left:** CGATGACCTGCCTTC

**SEs_inactive_strand5_right:** GTTACTGTTTAGCCTGCTCTACCAGAC

**SEs_activation:** GGTTACTGTTTAGCCTGCTCTA
/Cy3/ denotes a Cy3 fluorophore covalently attached to the 5’ end of DNA.

### 2.4.3. DNA nanopore and nanotube channel preparation

**Preparation of annealing solution for DNA nanopores**

To assemble DNA nanopores, 50 μl of an annealing mixture was prepared that contained M13mp18 scaffold, staple strands, and fluorescence attachment strands in TAEM buffer in the quantities shown below.

<table>
<thead>
<tr>
<th></th>
<th>Desired final concentration (nM or fold)</th>
<th>Stock concentration (nM or fold)</th>
<th>Volume added (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td></td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>TAEM buffer</td>
<td>1</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Staple mix (per strand)</td>
<td>200</td>
<td>1389</td>
<td>7.2</td>
</tr>
<tr>
<td>M13 scaffold</td>
<td>5</td>
<td>100</td>
<td>2.5</td>
</tr>
<tr>
<td>Fluorescence attachment strand mix</td>
<td>25</td>
<td>1000</td>
<td>1.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

**Preparation of annealing solution for DNA nanopores with adapters**
To allow DNA monomers to assemble on DNA nanopores to form nanotube channels, 24 adapter strands were added to the nanopore structures as they were assembled. The design and sequences of the adapter strands added to the nanopores are the Adapter A strands described in Jia, S. et al\textsuperscript{101}. 50 μl annealing mixture consisting of M13mp18 scaffold, staple strands, adapter strands, and fluorescence attachment strands in TAEM buffer were prepared in the quantities shown below.

<table>
<thead>
<tr>
<th></th>
<th>Desired final concentration (nM or fold)</th>
<th>Stock concentration (nM or fold)</th>
<th>Volume added (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td></td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>TAEM buffer</td>
<td>1</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Nanopore staple mix</td>
<td>200</td>
<td>1389</td>
<td>7.2</td>
</tr>
<tr>
<td>Adapter A strand mix</td>
<td>100</td>
<td>1000</td>
<td>5</td>
</tr>
<tr>
<td>M13 scaffold</td>
<td>5</td>
<td>100</td>
<td>2.5</td>
</tr>
<tr>
<td>Fluorescent attachment</td>
<td>25</td>
<td>1000</td>
<td>1.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

**Annealing protocol**

Seeds were annealed by running the following thermal schedule on the prepared annealing mixture with a thermocycler (Eppendorf Mastercycler):

1. 5 mins at 90°C
2. 90°C to 45°C at 1°C/min
3. 45°C for 60 mins
4. 45°C to 37°C at 1°C/10mins
5. 37°C hold until sample retrieval

**Nanopore purification and fluorescent labeling**

After thermal annealing, DNA nanopores without adapters were purified using 100kDa Amicon 9lta-0.5mL centrifugal filter units (Millipore Sigma, UFC510096). The concentration of purified seeds was measured by adopting the method developed by Agrawal et al\textsuperscript{103}. After purification, an imaging solution was prepared by mixing 0.3 μl seed solution with 19.7 μl tile mix solution (containing 0.05 mg/ml BSA). 6 μl mixture was then transferred to a glass slide to be imaged under a fluorescence microscope with 60x objective. We continued to dilute the purified seeds until 100-200 seeds per field of view (87 μm x 87 μm) were observed, indicating an approximate seed concentration of 6 pM in the imaged solution.

The final concentration of the purified nanopores was measured to be about 1 nM. The nanopores used to assemble nanotubes were purified using the same filter units but were concentrated during the purification process to a final concentration of 2 nM. Specifically, 100 μl of seed solution and 300 μl TAEM buffer were added to a filter unit and centrifuged at 3000 RCF for 4 min in a fixed-angle centrifuge. The sample was washed two more times by adding 300 μl TAEM buffer into the remaining solution and repeating centrifugation. The purified seed solution was then collected by spinning the inverted filter in a new tube. In both cases, 0.15 μl of 100 μM ATTO647 labeling strand was added to approximately 40 μl purified seeds collected from the filter unit and was incubated at room temperature for 15 minutes at room temperature.
Assembly of nanotubes

To assemble DNA monomers into nanotubes attached to DNA nanopores, DNA monomers were first annealed separately and then mixed with purified nanopores prepared as described above. The annealing was performed by first preparing 20 μl solution containing 400 nM of each of the inactive SEs monomer strands (as listed in 2.4.2) in TAEM buffer and then annealed the mixture using the annealing protocol. 20 μl of the purified nanopores were then mixed with the prepared 20 μl annealed inactive SEs monomers and 0.2 μl of a 50 μM solution of activation strand. The resulting solution was then incubated at 37°C for 3-5 hours to allow the nanotubes to grow.

Hydrophobic modification

To functionalize DNA nanopores with hydrophobic moieties, 1 μl DNA-cholesterol conjugate (“cholesterol strand” in Supplementary Note 1) at 10 μM concentration was added to either 40 μl nanopores without adapters after fluorescent labeling or 40 μl DNA nanotube channels. The solution was then incubated for 10 minutes at room temperature.

Figure S2.3. A coarse-grained model of DNA nanopore. The hairpin domains of the staples were not included to create the model. The DNA strands are shown using a space-filling representation in which each element is represented with a different color. a) Side view of the cylindrical barrel. b) Top view
down the axis of the cylinder. The yellow line spans the cylinder’s inner diameter. The predicted inner diameter of the cylinder was determined by averaging the lengths of this and 39 other lines across the cylinder’s interior starting at different positions along the helix. The mean inner diameter determined in the model using this method was 7.3 nm ± 0.4 nm (the uncertainty is the 95% confidence interval of the measurements in this article unless stated otherwise). We expect the inner diameter of the nanopore to change slightly over time due to thermal fluctuation.

Figure S2.4. Example fluorescence micrographs of DNA nanotube channels attached to DNA pores. The nanotubes (Cy3, green) and pores (ATTO647, red) were prepared as described in “Assembly of nanotubes” except that no DNA-cholesterol were added. The lengths of the nanotubes were measured by drawing segmented lines along the nanotubes in the images using ImageJ software. Nanotubes were not visible on 13% ± 3% (95% confidence interval) nanopores in the fluorescence images. Scale bar, 2 μm.
2.4.4. Sample preparation for transmission electron microscopy

Nanostructure samples were deposited on a formvar/carbon film support grid (Cat# FCF400-Cu, Electron Microscopy Sciences, Hatfield, PA, US) to be imaged. To prepare samples of DNA nanopores, nanotube channels, caps, and capped nanopores, 10 µl of the corresponding structures were prepared without attached DNA-cholesterol conjugates in TAEM buffer, and then were directly used to prepare the grids. For transmission electron microscopy (TEM) imaging of nanopores on SUVs and nanotube channels on SUVs, SUVs were first prepared and diluted as described in the Methods. The nanopores or nanotube channels were prepared with attached DNA-cholesterol conjugates in TAEM buffer. To prepare nanopores on SUVs, 7.5 µl nanopores were then mixed with 2.5 µl SUVs. To prepare nanotube channels on SUVs, 8 µl nanotube channels were then mixed with 2 µl SUVs. These mixtures were each incubated at room temperature for 10 minutes before use for preparing the grids.
Figure S2.5. Additional TEM Images of DNA origami nanopores without added DNA-cholesterol conjugates. Scale bar, 20 nm.

Figure S2.6. Additional TEM Images of DNA nanopores interacting with SUVs. Scale bar, 50 nm.
**Figure S2.7.** Design of the DNA channel cap.

Schematic of the DNA channel cap’s structure (The arrangement of its staples on the origami scaffold) produced using CADNano\textsuperscript{104} software. The cap is shown without the adapters that allow the cap to bind to a nanotube or a DNA nanopore. The staples in red have the same sequences as the corresponding staples in the origami seed. The 12 staples in green, whose sequences are listed in Table S2.3, are arranged to create a narrow neck in the structure.

**Table S2.3.** DNA cap staple sequences.

We designed the DNA cap by replacing 12 staple strands in the DNA origami seed structure in Mohammed & Schulman\textsuperscript{89} while preserving the other 60 staple strands.

<table>
<thead>
<tr>
<th>Name</th>
<th>Replaced staple name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>stp_47_1</td>
<td>T_5R12E_CYC_HP</td>
<td>CTCAGAGCGAGGCATAGGCTCCGCTTTTGCAGCCCTTGTAAAGAGCCGCCACC</td>
</tr>
<tr>
<td>stp_47_2</td>
<td>T_5R2E_HP</td>
<td>GAGAATAGGTCACCAGCCGAACCGTTTTTGCTCCGTTTACAAACTACAGGTAG</td>
</tr>
<tr>
<td>stp_47_3</td>
<td>T_5R4E_HP</td>
<td>AAAGGCGCTCCAAACCCGTGGGCTTTTGCACCAGGTTGGAGCCTTTCATTAC</td>
</tr>
<tr>
<td>stp_47_4</td>
<td>T_5R6E_HP</td>
<td>GCGAAACAGGAGGTGCTGCTGCTGCTTTGCAGCACCTTTTGAGGACTTACCAAGC</td>
</tr>
<tr>
<td>stp_47 _5</td>
<td>T_5R8E_HP</td>
<td>CCAATCATTTAGACGCTGGCTTTTGCCAGCGTTTCCGGAA CGAGGGAGTT</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>stp_47 _6</td>
<td>T_5R10E_HP</td>
<td>AAAGATTCTAAATTGGCAAGGACTTTTGTCCGTTCGCTTTGAGA AGGGAGTT</td>
</tr>
<tr>
<td>stp_79 _1</td>
<td>T_3R12E_CYC_ HP</td>
<td>CCCTCAGATCGTTTACCAGCTTTGCTTTTCGAAAGCTTCAGACGAC TACCAGCA</td>
</tr>
<tr>
<td>stp_79 _2</td>
<td>T_3R2E_HP</td>
<td>TGCTAAACTCCACAGAGCAGCTTTTGCACTTGAGTCGCTTTGCAGCC CTAAATAG</td>
</tr>
<tr>
<td>stp_79 _3</td>
<td>T_3R4E_HP</td>
<td>ATATATTCTCAGGTTCGCCGCTTTGCGGACGGTTCGGTTTGGAGT CATCAAG</td>
</tr>
<tr>
<td>stp_79 _4</td>
<td>T_3R6E_HP</td>
<td>CTATCATGGGAAGGGTTTCCCGATGGCTTTGCCATCCGTTCATTAA AATAAA</td>
</tr>
<tr>
<td>stp_79 _5</td>
<td>T_3R8E_HP</td>
<td>AGTAATCTTCATAAGGTCTGGTCTTTTCCGACCATGGATGACCAAG AGTAATCGG</td>
</tr>
<tr>
<td>stp_79 _6</td>
<td>T_3R10E_HP</td>
<td>ACGAATATTAATCATGGCAACCTGTTTTCCAGGTGGTTTGTGAATT TGGGATTT</td>
</tr>
</tbody>
</table>

**Figure S2.8.** A structural snapshot from a coarse-grained model of the DNA cap. The model was generated with oxDNA\textsuperscript{104} program using an initial configuration consisting of the PDB generated by CADNano\textsuperscript{45} (Supplementary Figure 2). A Monte Carlo simulation of 104 steps was first performed to relax the initial configuration. A molecular dynamics simulation of 2*108 steps was then performed to relax the configuration toward equilibrium. External forces in the form of mutual trap were used during the simulation to facilitate the wrapping of the configuration into a cylindrical structure.
Figure S2.9. Additional TEM Images of DNA origami caps. Scale bar, 20 nm.
Figure S2.10. Example wide-field fluorescence image of DNA caps bound to DNA nanopores. The pores labeled with ATTO647 (red) were mixed with two-fold concentration of caps labeled with ATTO488 (green). The mixture was incubated at room temperature for 3 hours before being imaged on a glass coverslip. The pores were considered capped if the centers of the pores and the caps were within 5 pixels (1 pixel=168 nm). 97.3 ± 0.6% (SD, N=678) pores were capped.

2.4.5 A different design of DNA nanopores

Before we developed the DNA nanopore design as shown in Fig 2.1, a different design approach was used to develop DNA nanopore and nanochannel. Adapting the funnel-shape DNA nanopore design reported by Göpfrich et al\textsuperscript{53}, we modified the DNA origami structure by removing the cap region and accommodating a partial structure of DNA nanotube seed\textsuperscript{89}, which
we then named the seed pore. The structure of the seed pore was visualized through a coarse-grained simulation\textsuperscript{105} (Fig. 2.12). The seed pore then could be used to template the growth of DNA nanotube channels for investigating the molecular transport through the channel.

\textbf{Figure S2.11.} Design of the DNA seed pore. Produced using CADNano\textsuperscript{104} software.
Figure S2.12. Simulated structure of the seed pore. Visualized in UCSF Chimera. The structure consists of a 12 helical transmembrane stem (bottom right region), which was extended to have a nanotube seed structure (top left).

However, when we verified the seed pore structure using TEM imaging, the images suggested the seed pores were not properly formed (Fig. S2.12). Most of the formed seed pores showed a bending at the middle of the structure, indicating a flaw in the DNA origami design of this structure, but the root cause of this bending has not yet been found. Thus, the seed pore structure was not used in the molecular transport studies.
Figure S2.13. TEM image of DNA seed pores assembled in TAEM buffer. The orange circles point to several examples of seed pore structures that are not formed as designed.
3.1 Introduction

Rather than using complicated natural membrane systems, such as cellular membranes and secretory vesicles, that have many biological components, various simplified model membrane systems have been developed to retain the lipid bilayer structure. Planar lipid bilayers, and different sizes of lipid vesicles and polymers are commonly used to study the properties of protein channels and synthetic nanopores.

Planar lipid bilayers are usually used in electrophysiological studies of membrane channels, in which the transport rates of ions across the membrane through inserted channels are monitored in a well-controlled artificial environment. Specifically, a chamber with cis- and trans- compartments are connected through a small aperture and a planar lipid bilayer can form on the aperture when a lipid solution is added. An electrochemical potential is then applied across the membrane after channels incorporate into the membrane. The ionic current through the channels are measured and channel conductance is obtained. This method enables measurements of transport within single channels and individual translocation event. The lipid composition and chemical conditions can also be easily controlled in the planar bilayer setup. However, this method requires an intricate electrophysiological setup, and the planar bilayer lacks mechanical and long-term stability.

Lipid unilamellar vesicles, consisting of a single lipid bilayer, mimic the enclosing lipid membranes in nature has been widely used to a variety of biological processes, including transmembrane transport. Lipid unilamellar vesicles are categorized into small unilamellar
vesicles (SUVs) of tens of nanometer diameters, large unilamellar vesicles (LUVs) of up to 1 micrometer diameters, and giant unilamellar vesicles (GUVs) of tens of micrometer diameters. They also show advantages such as ease of large-scale formation and enabling highly parallel measurements. GUVs are particularly of interests in many studies since they provide a cell-like structure with similar membrane curvature and can be directly observed using optical microscopy.\textsuperscript{108,112,113}

Several methods have been developed to produced GUVs, including gentle hydration, electroformation, inverted emulsion, microfluidic jetting and more.\textsuperscript{114} The gentle hydration method was firstly developed to prepare GUVs\textsuperscript{115}, which consists of the dehydration of lipid components and a rehydration process in the desired buffer. To enhance the GUV formation efficiencies and to overcome the ionic condition limitations, the gel-assisted hydration method was developed.\textsuperscript{116–119} Specifically, the lipids dissolved in the organic solvent is spread on a thin dried film of agarose or polyacrylamide. After solvent evaporation and hydrating the dry film with buffer, the GUVs then rapidly form at the interface between the swollen gel and the buffer.

Many recent studies used GUVs to investigate the functions of transmembrane proteins, such as the protein insertions\textsuperscript{107}, voltage gating of ion channels\textsuperscript{120}, protein distribution in membranes\textsuperscript{121}. GUVs are also commonly used in the studies of DNA-based nanopores as a tool for bulk measurements of transport rates of fluorescent molecules through the nanopores incorporated in the vesicle membranes.\textsuperscript{50,52,122} However, the behaviors of molecular transport within these nanometer scale DNA nanopore channels are less studied. Whether the surface effects of the channels played an important role in the molecular transport is still not well understood.
In chapter 2, we constructed a microns-long nanochannel of a large diameter through self-assembly. In this chapter, we seek to investigate the transport rates of small molecules through the DNA nanopores and nanochannels we designed (Fig. 3.1a). We use a dye influx assay to measure the kinetics of the diffusive transport of a small fluorescent dye across the membranes of GUVs (Fig. 3.1b). Analysis of the transport kinetics with a diffusion model of end-to-end transport reveals a diffusion-based transport mechanism through the nanopore. We then show DNA nanochannels of the same diameter and microns lengths, self-assembled on the DNA origami nanopores from DAE-E double crossover DNA tiles, also induce diffusive transport of small molecules after membrane insertion. A DNA origami cap that closes the channel opening, which eliminates channel-mediated transport, demonstrating that transport through the channel occurs dominantly end-to-end rather than across channel walls (Fig. 3.1c).

Our study thus shows that DNA nanochannels can mediate transport from one end of a barrel to the other at distances ranging from tens of nanometers to microns. The rates of transport in these channels are consistent with those in bulk solutions, allowing for precise design of flow rates and a DNA origami cap that hybridizes to the structure can completely halt cross-channel transport. These advances suggest a toolkit of self-assembling elements for nanoscale fluid transport networks. Therefore, with recent advancements in DNA bioconjugations, these nanotubes are potential components in building complex nanoscale devices with applications as smart drugs and biosensors.
Figure 3.1. Scheme for studying end-to-end transport of small molecules through micron-length self-assembled DNA nanochannels. **a)** End-to-end transport of small molecules through micron-length channels could allow for controlled transport in such channels between cells, synthetic microreactors, droplets or other entities. Molecules must diffuse from one end to a channel to another rather than across the channel walls to prevent serious loss during transport. **b)** Assay used to characterize the rates of transport through a microns-long channel. When the channel attached to a nanopore enters lipid membranes of a giant unilamellar vesicle (GUV), fluorescent dyes diffuse into the vesicle due to the concentration gradient across vesicle membranes; the channel and pore allow equilibration of the concentration of fluorescent dyes. **c)** A cap that binds to and plugs the opening of the nanotube channel would prevent transport from the opening at the channel’s end into the vesicle but not transport across the channel walls.
3.2 Methods

Materials

96-well glass-bottom plates with a streptavidin coating were purchased from Arrayit (Sunnyvale, CA, USA. Cat. No. M96S). 5(6)-Carboxytetramethylrhodamine (TAMRA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All lipids were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Tetramethylrhodamine isothiocyanate–Dextran (TRITC-Dextran) of average molecular weight of 4,400 Da was purchased from Millipore Sigma (St. Louis, MO, USA. Cat. No. T1037).

GUV Preparation

The protocol for preparing GUV was adapted from Horger et al.\textsuperscript{116} with modifications. 1% (w/w) agarose in deionized water was boiled in a microwave oven. 200 µl of the warm agarose solution was poured onto a glass-bottom dish. The dish was kept on a hotplate at 80 °C for an hour to form a dry agarose film. 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) lipids and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(biotinyl) (Biotinyl PE) were dissolved in chloroform to a concentration of 5 mg/ml in glass vials. A fluorescent lipid, 1-palmitoyl-2-(dipyrrometheneboron difluoride)undecanoyl-sn-glycero-3-phosphocholine (TopFluor® PC), was dissolved in chloroform to a concentration of 1 mg/ml in a glass vial. The three lipid solutions were mixed in chloroform to prepare a 1 mg/ml lipid mix solution with a composition of 88% DPhPC, 10% Biotinyl PE, and 2% TopFluor PC. 40 µl lipid mix solution was then deposited onto the dry agarose film on the dish. The dish was kept in a vacuum desiccator overnight to allow the lipid solution to completely dry. After solvent was removed,
300 µl of 0.2 M sucrose solution was added to the film in the dish. The dish was kept undisturbed at room temperature for 2 days to allow GUV formation.

**Dye influx assay setup**

90 µl TAE buffer supplemented with 0.2 M sucrose and 309 nM TAMRA was added into a well on a 96-well glass-bottom plate with a streptavidin coating. 6 µl GUV solution was then added into the well. The GUVs encapsulating 0.2 M sucrose settled down onto and became immobilized on the glass surface in about 5 minutes due to density differences between the solutions inside and outside the GUVs and biotin-streptavidin binding between the vesicles and the glass surface. 35 µl of solution containing either nanopores, capped nanopores, nanotube channels, or capped nanotube channels prepared in TAEM buffer, after cholesterol modification, were mixed with 9 µl 1 M glucose before being added into the well to maintain a final concentration of 0.2 M glucose. The final concentration of magnesium ions was 3.1 mM.

The sample on the glass-bottom plate was imaged on an inverted confocal microscope (Nikon Ti2-E with A1 confocal unit) using a 60x/1.49 NA oil immersion objective lens. DNA seed pores were imaged using a 640 nm diode laser, TAMRA and DNA nanotubes were imaged using a 560 nm diode laser, and GUVs were imaged using a 480 nm diode laser. All three fluorescent channels at one focal plane position in a large area of 4 x 4 fields of view were captured with 60- or 71-second time intervals. Each field of view had dimensions of 512 x 512 pixels, corresponding to a physical field-of-view size of 210 µm x 210 µm. The experiments with DNA seed pores were observed for a total time length of 210 minutes and experiments with DNA nanotubes were observed for a total time length of 8 hours. The Nikon Perfect Focus System (PFS) was used to eliminate axial focus fluctuations.
Fluorescence image data processing

In order to quantify the fluorescence intensities of TAMRA over time inside and outside GUVs observed using confocal microscopy, we first used ImageJ software to identify and track individual GUVs captured. The outlines of GUVs at each time point were identified by applying the “thresholding” function in ImageJ software to convert the grayscale images of GUVs into binary images. The image areas inside GUVs at each time point were identified by selecting the areas separated by GUV outlines that had circularities greater than 0.65 using the “Analyze Particles” function in ImageJ software. Similarly, one area outside GUVs at each time point was identified by selecting the largest area in the image with circularity smaller than 0.6. The mean fluorescence intensities of TAMRA in the areas inside and outside the identified GUVs at each time point were measured from grayscale images of TAMRA. The TAMRA concentration fractions of individual GUVs at each time point were calculated by dividing the mean intensities inside GUVs to the mean intensity outside GUVs.

3.3 Results and Discussion

To determine whether nanotube channels could direct end-to-end transport of a small molecule (Fig. 3.1a), we designed a scaffolded DNA origami cap that binds to the open end of a DNA nanopore or nanotube via sticky-end hybridization by modifying a rigid cap structure for nanotubes (chapter 2). Pore-assisted transport through capped channels could therefore only happen if the small molecule could readily diffuse transport across the channel’s wall, i.e. through interhelix gaps or defects in the nanotube’s structure. We thus hypothesized that by comparing rates of pore-mediated small molecular transport across capped and uncapped
channels, we could determine whether nanotubes could direct end-to-end transport of small molecules across their full lengths without loss through channel walls.

Transport through microns-long channels would be difficult if the small molecules moving through channels proceeded primarily through interaction with the channel surface or if these molecules aggregated to form clumps that could obstruct the channel. We therefore first sought to understand whether molecular transport within the DNA nanotube channel followed macroscopic descriptions of diffusion by monomeric species or whether the nanofluidic surface effect was important, by measure the rate of small molecule transport through the channels and determining whether these rates were consistent with the predictions of classical diffusion laws\textsuperscript{123,124}. We measured the flux of a fluorescent dye, carboxytetramethylrhodamine (TAMRA) through DNA nanopores into giant unilamellar vesicles (GUVs). TAMRA is a member of the rhodamine fluorophore family and was selected for its small size, neutral charge, and very low membrane permeability\textsuperscript{125}. These properties suggested TAMRA not to interact with DNA structures or diffuse through lipid membranes. TAMRA dimerization is also limited under our experimental conditions. We developed a dye influx assay in which GUVs were immersed in a TAMRA solution containing DNA pores and the rates of TAMRA influx into GUVs over time were quantified using time-lapse microscopy.

We first measured how much the presence DNA nanopores changed the rate of TAMRA transport into GUVs. Biotinylated, fluorescently labeled GUVs encapsulating a sucrose solution (Methods) were first added to a glucose solution supplemented with TAMRA dye to create a TAMRA concentration gradient across the vesicle membranes. The experiments were performed in a streptavidin-treated glass-bottom well; the GUVs sank down onto the glass coverslip due to density gradients, where they were immobilized by biotin-streptavidin linkages, allowing time-
lapse observation of individual vesicles. We then added fluorescently labeled DNA pores to a final concentration of 1 nM and monitored the fluorescent intensities inside and outside GUVs for 3.2 hours with a confocal microscope. The pores and membranes colocalized within 10 minutes, suggesting the cholesterol-functionalized DNA pores could interact with membranes to enable transport of TAMRA across the membrane. In experiments with pores, TAMRA entered a large fraction of GUVs at rates far above those observed in control experiments with GUVs without pores (Fig. 3.2 a-c, g), demonstrating that the designed pores could facilitate transport across lipid membranes.

**Figure 3.2.** TAMRA transport through open and capped DNA origami nanopores. a. Assay of dye influx rates through DNA nanopores into GUVs. b. Fluorescence micrographs of an example GUV during TAMRA influx as in a. Top row: composite images of TAMRA (red), vesicle membrane (green), and DNA nanopores (cyan). Middle row: TAMRA fluorescence. Bottom row: DNA nanopore fluorescence. c. Representative traces of fractional fluorescence intensities of GUVs during experiments shown in (a) and (e). Pore-mediated influx begins after a lag period. Capped pores do not mediate influx. Regression curves of fractional intensities are best fits to Equation 1 (Supplementary 3.4.1). d. The probability distribution of GUV influx rates with added DNA pores (N = 418) and capped DNA pores (N = 264).
Assay of dye influx rates through capped DNA nanopores into GUVs. f. Fluorescence micrographs of an example GUV during little TAMRA enters the vesicle. Fluorescent labeling as in (b) except DNA caps (green) are also in the top-row micrographs. g. Fractions of GUVs with influx rates above 5 µm³/min with added (left to right) nanopores, nanopores without cholesterol-DNA conjugates (N = 165), cholesterol-DNA conjugates (N = 114), nanopores and 500-kDa TRITC-dextran dyes in place of TAMRA (N = 176) and capped nanopores. Error bars are 95% confidence intervals. h. Cumulative probability distributions of influx rates into GUVs tracked in the nanopore, capped nanopore, and control experiments in (g).

To quantify influx rates, we wrote software to automatically identify 427 GUVs and measured the intensities of fluorescence inside them over time. To use these fluorescence values to measure influx rates, we computed the fractional fluorescence intensity (FFI) inside each vesicle, which we defined as the ratio of the mean fluorescence intensity inside a vesicle to the mean fluorescence intensity outside of vesicles in a given field of view. The fractional fluorescence intensity of many vesicles initially increased slowly but then suddenly increased dramatically. The initial rates of FFI increase were similar to the rates the FFI of vesicles in control experiments increased (Fig. 3.2c), suggesting these initial increases were due to TAMRA slowly permeating through the GUV membranes and preceded the insertions of a DNA pore or pores into the membranes. Although pores adsorbed to membranes rapidly, this initial phase GUVs often lasted >1 h, suggesting that the rate of insertion of DNA nanopores is low. A high energy barrier to insertion of DNA origami nanopore into GUV membranes has been observed previously.58,126

The rapid FFI increases we observed only when the DNA pores were present therefore reflected the flux of TAMRA across the membrane mediated by the DNA pores. We hypothesized that the DNA pores facilitated TAMRA influx into GUVs by forming a channel.
with the pore’s inner diameter through which TAMRA could passively diffuse and that the contributions of channel surface interactions were minimal. If this were the case the rate, a concentration gradient would form across each pore and the flux of TAMRA into GUVs should follow Fick’s law of diffusion.

To verify this hypothesis, we developed a model that accounted for both fast, pore-mediated influx and slow influx across the membrane. Fast TAMRA influx was modeled as net diffusion of TAMRA from the bulk solution outside the vesicle into a finite compartment through DNA pore channels (Supplementary 3.4.1). A DNA pore was modeled as a rigid cylindrical channel with non-permeable walls. Fick’s law predicted that the flux of TAMRA at a given time should be linearly proportional to difference in concentrations inside and outside the pore and to the total cross-section area of the inserted channels. Slow, non-pore mediated TAMRA influx was modeled as a linear increase in fractional intensity (Supplementary 3.4.1 Equation 8). In the resulting model of FFI increase,

\[
f(t) = \begin{cases} 
  f_0 + a \times t \\
  f_0 + a \times t + (1 - f_0 - c \times t_1) \left[1 - e^{-\frac{(t-t_1)}{\tau}}\right]
\end{cases}
\]

when \( t < t_1 \)

\[
f(t) = f_0 + a \times t + (1 - f_0 - c \times t_1) \left[1 - e^{-\frac{(t-t_1)}{\tau}}\right]
\]

when \( t \geq t_1 \) (1)

where \( f(t) \) is FFI at time \( t \), \( f_0 \), \( a \), \( \tau \), and \( t_1 \) are constant parameters.

Non-linear regression produced good fits of the FFIs of most vesicles with Eq. 1; the small fraction of GUVs where the FFI experienced bursts, possibly due to lipid membrane defects\(^{127}\), or multiple rapid increases were excluded. We used these fits to calculate the fast influx rate \( (k_f = \frac{V}{\tau}) \) that was pore mediated, where \( V \) is vesicle volume, and the background influx rate \( (k_b = a \times V) \) for each vesicle (Supplementary 3.4.1). We also obtained the initial FFI \( (f_0) \) and the time when fast influx starts \( (t_1) \).
Because DNA origami pores each have the same lengths and diameter, the amount of pore-mediated influx contributed by each pore should be the same. The observation that most GUVs showed a single increasing curve in fractional intensities after the lag time indicated that either only one nanopore insertion occurred on a GUV or the insertions of multiple pores occurred at the same time rather than sequentially. One hypothetical mechanism by which multiple simultaneous insertions could occur was that instead of directly inserting onto the membranes of GUVs, the DNA pores preferred to insert on small vesicles, which were also present in the GUV solution as byproducts during GUV preparation, with a faster insertion rate due to the low membrane curvatures of small vesicles. The pores were then transferred onto GUVs through vesicle fusions as vesicle fusions could frequently occur in solution. Thus, if the pore-mediated influx into individual vesicles was induced by a discrete number of pores, the influx rates observed should be quantized rather than continuously distributed with each quantum being the rate when a specific number of nanopores is inserted into different vesicles. In this case, we would be able to determine the number of inserted pores that induced influx on each GUV by analyzing the influx rates for a population of GUVs.

To determine whether the influx rates were quantized, we averaged the posterior distributions of pore-mediated influx rates (to include measurement error) for the GUVs found in nonlinear regression to produce a composite probability distribution over influx rates (Fig. 3.2). The resulting distribution had a series of peaks. The Fourier transform of this distribution over 15 – 120 μm³/min had a dominant period of 13.1 ± 1.5 μm³/min (Fig. S3.5). This measured value is very close to the first peak in the distribution (12.4 μm³/min). Together these results suggest that each pore mediated influx of 12.5-13.5 μm³/min. which corresponds to a net TAMRA flux
through each nanopore of $40.5 \pm 4.6$ molecules per second for the 309 nM TAMRA used in experiments.

The rate of TAMRA influx through a single nanopore that would be expected because of Fickian diffusion is $14.7 \pm 1.8 \, \mu m^3/\text{min}$ (Supplementary 3.4.7), almost precisely the measured rate. Although electrostatic interactions, van der Waals forces between solutes and channel entry effects can play important roles in molecular transport within nanoscale channels, the consistency between the measured TAMRA transport rates and the rates predicted by the continuum diffusion model suggested that the diffusion of TAMRA, an uncharged small molecule, in a DNA nanopore resembles diffusion in bulk solutions.

The flux rates for many GUVs, however, were much lower than the calculated flux rate due to a single nanopore. These small influx rates were likely the result of leaky transport caused by inherent membrane defects rather than induced by nanopores. To differentiate GUVs that had pore mediated influx from the ones with only baseline membrane leaks, an influx rate threshold was needed for calculating the fraction of GUVs with significant influx above baseline. Given the theoretical single-pore flux rate, we set this threshold at $5 \, \mu m^3/\text{min}$, which subsequently showed 49% of all GUVs monitored in the experiment with DNA nanopores had effective influx above this threshold. The percentages of GUVs with effect influx were significantly lower in the control experiments (Fig. 3.2g) and could be due to transient holes appeared in GUV membranes. The cumulative probability distributions of influx rates also suggested that effective influx into vesicles occurred only when cholesterol modified nanopores were present (Fig. 3.2h, Supplementary S3.3). Further, to investigate whether the transport through the pore is size-selective, we used the same concentration of a 500-kDa dextran conjugated dye, tetramethylrhodamine isothiocyanate-dextran (TRITC-dextran), which had a
hydrodynamic radius of 15.90 nm,\textsuperscript{132} instead of TAMRA dyes in the influx experiment. As expected, a much lower percentage of GUVs (15\%) had effective influx during the experiment.

Pores could mediate transport by allowing TAMRA to diffuse from one end of the nanopore channel to the other or by allowing TAMRA to diffuse through gaps in the channels' helical walls\textsuperscript{133}. To determine whether TAMRA diffuses across channel walls, we measured the amount of the amount of flux mediated by nanopores bound to DNA origami caps. We assembled capped pores by mixing caps at two-fold excess with nanopores. Fluorescence micrographs revealed that 97.3\% ± 1.2\% of nanopores were bound to caps (Fig. S2.10). Capped nanopores localized to vesicle membranes, but TAMRA flowed faster than 5 \(\mu\text{m}^3/\text{min}\) into only 3.0\% ± 2.1\% of GUVs, fewer than the percentage in control samples (Fig. 3.2d-h). TAMRA therefore moves through pores via end-to-end diffusion and not through pore walls.

The experiments above suggested that TAMRA diffusion through channel walls was minimal, and pore-mediated transport of TAMRA occurred almost entirely via diffusion of TAMRA into a nanopore’s open end. To investigate whether TAMRA could also move through a much longer nanochannel without diffusing through the channel’s walls, we prepared DNA nanotube channels by growing DNA tile nanotubes from nanopores that presented complementary sticky ends. 87\% ± 3\% of nanopores had nanotubes (Fig. S2.4); the nanotubes had broad a range of lengths, and on average were 797 nm ± 57 nm) (Fig. 3.3c). To characterize TAMRA transport through these nanotube channels, the DNA nanotube channels grown from 1 nM nanopores were modified with cholesterol and added to GUVs in a TAMRA solution. There were sharp increases in the fractional intensities inside many of the 167 tracked vesicles after initial lag periods (Fig. 3.3a-b), implying that like the DNA nanopores, DNA nanotubes mediated TAMRA flux across vesicle membranes.
Figure 3.3. Rates of dye diffusion through DNA nanotube channels into GUVs suggest dye diffuses end-to-end through nanotube channels. **a.** Schematic representation of the dye influx assay used to measure rates of TAMRA transport through DNA nanotube channels into GUVs. **b.** Fluorescence micrographs of an example GUV at different time points showing TAMRA influx into the vesicle. Fluorescent labeling is as described in Fig. 3b besides that DNA nanotubes (red) are in the same fluorescent channel as TAMRA but have higher intensities. The increases in vesicle size could be a result of a small imbalance in osmotic pressures and was taken into account when calculating influx rates. **c.** Histogram of DNA nanotube lengths measured in fluorescence images (Figure 3.4). The last bin accounts for nanotubes with lengths >2.4 µm. **d.** Representative traces of fractional fluorescence intensities of a GUV in the experiment depicted in (a). Influx speeds up abruptly after a lag time. A second trace shows an example GUV from the experiment depicted in (e) with no changes in flux rate. Regression curves are the best fits of the Fickian diffusion model to the experimental measurements (Supplementary 3.4.1). **e.** Schematic representation of the experiment used to measure transport through a capped nanotube channel. **f.** Fluorescence micrographs of an example GUV in the capped nanotube experiment with same fluorescent labeling as in (b) except DNA caps (green) in the first-row micrographs. **g.** Fractions of GUVs with fast TAMRA influx for the experiments in (a), (e) and in control experiments where nanotube channels lack
cholesterol modifications and with only cholesterol-DNA conjugates present but not channels. h.
Cumulative probability distribution of influx rates in the range of 0 – 10 μm³/min of GUVs tracked in the experiments in (g). Scale bars, 2 μm.

Eq 1 should model the rate of diffusive flux of TAMRA through nanotube channels. but the rates of flux through nanotubes should be slower compared to the pore mediated influx rates because the molecular flux through a channel should be inversely proportional to the channel’s length (Supplementary 3.4.1). Because the number of GUVs tracked was small compared to the number in the influx experiment with nanopores, to analyze the flux rates using as many measurements as possible, we included the GUVs that showed two sequential influx curves in the fractional intensity measurements by fitting a modified diffusion model that captured two separate insertions. Regression of the fluorescence measurements with the diffusion models did produce good fits, although, consistent with the prediction of decreased flux, the rates of channel mediated transport were close to the rate of leak transport across the membrane than in the case of DNA nanopores (Fig. 3.3d).

Based on the channel diameter and TAMRA diffusivity, the diffusion model suggested an influx rate of 1.0 μm³/min through a nanotube channel of average length. End-to-end transport within nanotube channels would result in a TAMRA influx rate of 1.0 μm³/min through a 797 nm long (average length) nanotube. Thus, we counted the number of vesicles that experienced influx rates over 0.8 μm³/min as the vesicles that had effective influx, which accounted for 54% ± 7% of vesicles. Less than 20% of vesicles control experiments in which nanotubes without cholesterol modification exceeded this level of dye influx (Fig. 3.3g). We then plotted the cumulative density distribution of the measured influx rates for vesicles (Fig. 3.3h,
Supplementary 3.4.6). The broader distribution of the influx rates measured in the nanotube channel experiment compared to the distributions from controls also confirmed that most vesicle influx was induced by inserted nanotube channels with cholesterol modification.

The median TAMRA influx rates for GUVs with effective influx in the dye influx experiment was 3.3 μm$^3$/min (N=59, 95% CI = [1.9, 5.7]), after excluding GUVs not fitting well in the regression. This median influx rate was larger than the theoretical rate of 1.0 μm$^3$/min induced by an average-length nanotube channel, which could be a result of the nanopores without attached nanotube channels present in the solution or insertions of multiple channel insertions onto single vesicles. Because the polydisperse lengths of the nanotubes channels would lead to different channel flux rates, we could not determine the number of inserted channels on each GUV, or single-channel flux rate based on the measured GUV influx rates.

The smaller flux rates of TAMRA through nanotube channels than through nanopores suggested that the TAMRA was diffusing across nanotube channel lengths, rather than, across channel walls. To determine the extent to which TAMRA diffused from one end of a nanotube channels to the other, we measured the extent to which capped nanotube channels could mediate TAMRA influx into vesicles. 94% ± 3% nanotube channels were bound to caps after incubating the channels with caps. When we used capped channels in the dye influx experiment, <20% of vesicles had levels of TAMRA influx consistent with channel mediated transport (>0.8 μm$^3$/min), indistinguishable from levels observed in control experiments (Fig. 3.3g). The cumulative distribution of the measured influx rates was also very close to the distribution of rates seen in these control experiments and very different from the distribution measured using uncapped nanotube channels (Fig. 3.3h). DNA channel caps essentially eliminated most TAMRA through the nanotube channels, implying that the dominant mode of transport of TAMRA flux mediated by nanotube channels was from one end of a nanotube channel to the other.
We sought to better understand the structural basis of TAMRA transport inside DNA nanotube channels using an all-atom molecular dynamics simulation of TAMRA diffusion within a DNA nanopore. We modeled a 25 nm-long segment of the DNA origami nanopore, explicit solvent, and TAMRA molecules both inside and outside the pore at the beginning of the simulation. In 70 simulated nanoseconds, TAMRA molecules inside the channels diffused readily across the length of the pore segment, consistent with TAMRA diffusion within nanopore channel observed in experiments. Interestingly, several instances of TAMRA crossing the nanopore walls through the gaps between the DNA helices were also observed, indicating that transport across the nanopore boundaries could occur. One potential reason for the discrepancy between these simulations and the experimental observations is glucose binding to oligonucleotides, which can cause expansion of the DNA duplex diameter and reduced gap distances between DNA helices.\textsuperscript{134,135} The simulation could not capture these effects, which may be significant given 200 mM glucose was added to TAMRA solution to achieve osmotic balance between vesicle interiors and the bulk solution.

In summary, we have designed a self-assembled DNA nanochannels with micrometer-length that demonstrated transport of small molecules, as measured in dye influx assays. Fitting a continuum diffusion model to the measurements revealed that transport within the channel could be explained by Fick’s diffusion laws. In addition, the observation that DNA caps bound at the channel’s end prevented influx revealed transport within the DNA channels occurred end-to-end rather than across channel walls.

Therefore, the DNA nanochannels can be used to direct transport of small molecules in solution over micron-scale distances. These self-assembled nanochannels have been previously shown to be capable of self-repair (chapter 5) and can grow such that their endpoints specifically
attach at molecular landmarks, making them a promising structure to be used in assembling highly complex nanoscale devices for drug delivery and biosensing. We also expect the large channel diameter compared to many existing synthetic nanopores would allow transport of macromolecules such as proteins and DNA. After functionalization, the long channel lengths would also be advantageous at characterizing the DNA-protein or protein-protein interactions within the channel for the long residence time of protein molecules transporting through the channel. After functionalizing the channel interiors with antibodies or DNA aptamers the nanotube channels are potential tools for detecting and studying proteins of interest.

3.4 Supplementary Information

3.4.1 A bulk diffusion model of TAMRA influx into vesicles through DNA nanopores

We model the influx of TAMRA into the vesicles as diffusive transport of TAMRA molecules from a bulk solution into a compartment through DNA nanopores. The concentration gradient of TAMRA between the bulk solution and the compartment drives net diffusion (i.e. influx) of TAMRA into the vesicle. A pore is modeled as a rigid cylindrical channel of diameter \( d \) and length \( L \).

Because the volume of the bulk solution is much larger than the volume of the compartment, the TAMRA concentration in the bulk solution remains constant in the model. Complete influx of TAMRA into vesicles (so that the concentration inside a vesicle and in the bulk are approximately the same) takes half an hour to several hours. The time scale of mixing within the vesicle compartment is therefore much smaller than the time scale of transport from the bulk solution into the compartment. The solution in the compartment can thus be viewed as a uniform bulk phase (quasi-steady-state approximation). This assumption is consistent with our
observation in confocal micrographs that fluorescence intensities of TAMRA within the vesicles do not show spatial variations.

The molar flux of TAMRA \((J)\) through the channels into a vesicle is given by

\[
J = D \frac{C_{out} - C_{in}}{L}
\]

where \(D\) is the diffusion coefficient of TAMRA and \(C_{out}\) and \(C_{in}\) are bulk phase concentrations of TAMRA in the bulk phase outside and inside the compartment respectively.

The molar flow rate of TAMRA into a vesicle can be written as

\[
\dot{n} = J \cdot A = \frac{AD}{L} (C_{out} - C_{in})
\]

where \(A\) is the total of the cross-sectional areas of the channels spanning the membrane. The change in the amount of TAMRA inside the compartment is then given by

\[
\frac{dN}{dt} = \dot{n} = V \frac{dC_{in}}{dt}
\]

where \(N\) is amount (in moles) of TAMRA inside the vesicle and \(V\) is the volume of the vesicle. Equating (2) and (3) gives

\[
\frac{dC_{in}}{dt} = \frac{AD}{VL} (C_{out} - C_{in})
\]

Rearranging the equation to separate variables then gives

\[
\frac{dC_{in}}{C_{out} - C_{in}} = \frac{AD}{VL} dt
\]

We now introduce the fractional concentration \(f = \frac{C_{in}}{C_{out}}\), defined as the ratio of the TAMRA concentration inside the vesicle to the concentration outside. Substituting \(f\) into equation (6) gives

\[
\frac{df}{1-f} = \frac{AD}{VL} dt
\]
We can then solve for fractional concentration as a function of time by integrating the differential equation with the initial condition of \( f(t=t_1) = f_1 \) where \( t_1 \) is the time when the influx starts:

\[
    f_{\text{pore}} = f_1 + (1 - f_1)[1 - e^{-(t-t_1)/\tau}] \tag{7}
\]

Here we introduce the abbreviation, time constant \( \tau = \frac{V_L}{A_D} \) which has unit of min.

Equation (7) describes how fractional TAMRA intensity changes due to TAMRA influx through the pores, so this fractional influx is denoted as \( f_{\text{pore}} \).

“Leak” transport of TAMRA across the membrane (i.e., transport not mediated by nanopores) also occurs at rates described by equation (7). However, the leaky transport rate is much slower than the pore-mediated transport as observed in the dye influx experiments, so the time constant \( \tau \) for the leaky transport is very small. We use a linear approximation for the kinetics \( t \) during both the lag time and the influx phase, written as

\[
    f_{\text{leak}} = f_0 + a \cdot t \tag{8}
\]

where \( k_0 \) is the leaky influx rate of TAMRA into a vesicle.

Before a nanopore or nanopores insert into a vesicle \((t < t_1)\), the fractional concentration increases solely because of leak transport. During this period, the fractional concentration as a function of time is therefore

\[
    f = f_0 + a \cdot t \quad \text{when} \quad t < t_1 \tag{9}
\]

The fractional intensity when influx starts is therefore

\[
    f_1 = f_0 + a \cdot t_1 \tag{10}
\]

After nanopore insertion \((t=t_1)\), increases in the fractional concentration are attributed to both leak transport and pore-mediated influx. The fractional concentration as a function of time is therefore
\[ f = f_1 + a(t - t_1) + (1 - f_1) \left[ 1 - e^{-\frac{(t-t_1)}{\tau}} \right] \] when \( t \geq t_1 \) \hspace{1cm} (11)

Combining the two fractional concentration functions and plugging in the equation for \( f_1 \) results in a piecewise function for the fractional concentration:

\[
 f = \begin{cases} 
 f_0 + a * t & \text{when } t < t_1 \\
 f_0 + a * t + (1 - f_0 - c t_1) \left[ 1 - e^{-\frac{(t-t_1)}{\tau}} \right] & \text{when } t \geq t_1 
\end{cases}
\]

\hspace{1cm} (12)

3.4.2 Rate of TAMRA influx through a single DNA nanopore

The influx rate \( (k) \) of TAMRA into a vesicle through a single nanopore measured to be 13.1 ± 1.5 \( \mu \text{m}^3/\text{min} \) in experiments (see main text). To convert this rate into units of molecules per second, we plug \( k = \frac{V}{\tau} = \frac{AD}{L} \) into Eq. (4) and rearrange to write

\[
 \frac{dN}{dt} = V \frac{dC_{in}}{dt} = k(C_{out} - C_{in})
\]

Inserting \( k = 13.1 \pm 1.5 \ \mu \text{m}^3/\text{min} \), \( C_{out} = 309 \ N \), and \( C_{in} = 0 \) into (13), the net flux of TAMRA through a single nanopore at beginning of the influx process is calculated to be 40.5 ± 4.6 molecules per second.

3.4.3 A bulk diffusion model of TAMRA influx into vesicles through DNA nanotube channels

The DNA nanotube channels are cylindrical structures that have the same diameters as the DNA pores but longer lengths. We model influx of TAMRA through nanotube channels using the same model used for transport through DNA pores but adjust the channel length parameter. In this case, the fractional concentration of a vesicle as a function of time can be written as in equation (12).
For nanotube-mediated transport, the channel-mediated transport rates $k = \frac{V}{\tau} = \frac{AD}{L}$, should in general be smaller than the channel-mediated transport rates in for nanopore-mediated transport due to longer channel lengths ($L$).

Equation 12 is written to assume a single influx event but could be extended to account for multiple insertion events. Doing so would increase the complexity of fitting but would be required to properly fit influx curves in which there were multiple insertion events at different times. We had sufficient data from experiments with DNA nanopores to measure the distribution of influx rates and to deduce the influx rate through a single pore using only traces with single influx events. But experiments with produced too fewer traces with single influx to characterize rates of transport through open and capped nanotube channels. To measure the influx rates for eight vesicles in the dye influx experiments with the DNA nanotube channels that showed two distinct curves, we modified the diffusion model described in Supplementary Note 5 to account for influx events with two sequential insertions.

In the case of two sequential insertions, the fractional concentration as a function of time, $f(t)$, follows Equation 12 until an additional influx event starts at $t = t_2$, i.e.:

$$f = \begin{cases} 
    f_0 + a \ast t & \text{when } t < t_1 \\
    f_0 + a \ast t + (1 - f_0 - a \ast t_1) \left[ 1 - e^{-\frac{(t-t_1)}{\tau_1}} \right] & \text{when } t_1 \leq t < t_2 
\end{cases}$$

(14)

where $\tau_1$ is the influx time constant for the nanotube channels inserted at $t = t_1$. The influx rate after $t = t_2$ is then the sum of the influx rates due to leak transport, pore-mediated influx that starts at $t = t_1$, and pore-mediated influx that starts at $t = t_2$. Thus, the fractional concentration in the case where there are two insertions of a nanotube channel channels, at $t_1$ and $t_2$ respectively is
\[ f(t) = \begin{cases} 
  f_0 + a \cdot t & \text{when } t < t_1 \\
  f_0 + a \cdot t + (1 - f_0 - a \cdot t_1) \left[ 1 - e^{-(t-t_1)/\tau_1} \right] & \text{when } t_1 \leq t \leq t_2 \\
  f_0 + a \cdot t + (1 - f_0 - a \cdot t_1) \left[ 1 - e^{-(t-t_1)/\tau_1} \right] + (1 - f_2) \left[ 1 - e^{-(t-t_2)/\tau_2} \right] & \text{when } t > t_2 
\end{cases} \]  

(15)

3.4.4 Image analysis of fluorescence micrographs from dye influx experiments

In order to measure the changes in fluorescence intensities of hundreds of GUVs using fluorescence images captured over time during influx experiments, an image analysis algorithm was developed using ImageJ and MATLAB software.

The first step of the algorithm was to locate the vesicles and to determine their respective volumes. The 8-bit grayscale images of the vesicle membrane fluorescence channel and the TAMRA fluorescence channel were imported into ImageJ software as two time series stacks. The image stack of the vesicle membrane channel was converted into a binary image stack by taking the threshold values of 0-20 to find the outlines of vesicles in the images (Fig. S3.1). The “Analyze particles” function in ImageJ was then applied to the binary image stack of the vesicles, to find circular objects with specific diameters and extents of circularity in the stack. We excluded vesicles with either diameters smaller than 5 µm or extents of circularity less than 0.6 from analysis.

Because the 2-D circular cross-sections of the vesicles that appeared in the confocal fluorescence images at the specific height used were not necessarily the largest cross-sections of the vesicles, the diameters of the vesicle cross-sections in the images needed to be converted to the vesicles’ actual diameters (see 3.4.5). Because a 4 µm vesicle diameter in the confocal images corresponded to an actual vesicle diameter of 5 µm, a 4 µm diameter limit was used in the search criteria.
After the vesicles that fit the criteria were found in each slice, the vesicle size, coordinates, and the mean fluorescence intensities of TAMRA in the area inside the vesicles were measured and recorded by redirecting the measurements to the TAMRA channel image stack. This information was then used to measure the fluorescence intensities of TAMRA inside each vesicle and outside of the vesicles and to use these values to determine the fractional concentrations of TAMRA inside each vesicle over time.

The mean intensity in the TAMRA channel was used to determine the fluorescence intensity corresponding to the concentration of TAMRA added to the vesicles (the bulk concentration). The mean fluorescence intensity in the TAMRA channel outside the vesicles was obtained by using the “analyze particles” function over areas with less than 0.5 circularity. The fractional concentration inside a vesicle was then determined by dividing the measured intensity by this maximum intensity.

Because the vesicles were immobilized to the surface by biotin-streptavidin linkages, the vesicles showed minimal movement over time. Thus, each vesicle found in each slice was matched with the same vesicle across the stack based on its coordinates and size, so that the interior mean TAMRA intensities at each time point for each vesicle were obtained. The fractional intensity of each vesicle at each time point was then calculated by dividing the interior intensity by the exterior intensity at each time point. We excluded vesicles that burst or ruptured during the experiment by removing the vesicles that had an initial fractional intensity less than 0.5, an increase of over 0.1 in the fractional intensity within time interval of 1 minute or were observed in fewer than 60% of total time points.
**Figure S3.1.** Example fluorescence micrographs of GUVs from the dye influx experiment with DNA nanopores. a) Confocal fluorescence images in the vesicle membrane fluorescence channel were used to determine the vesicle locations within the images and their volumes. b) A binary image (red areas are retained after thresholding), whose on points contained the areas of the vesicles, was generated by using a threshold filter on the image in (a) that selected pixels with intensity values in the range of 0 – 20 pixel intensity values. The perimeters of each circle in the image were used to calculate the volume of the vesicle by assuming each vesicle had a spherical shape and using Equation 17 (section 3.4.5). The fluorescence intensities of TAMRA inside and outside the vesicles were measured by redirecting the intensity measurements to c), the grayscale fluorescence micrograph in the TAMRA channel. Scale bar, 10 µm.

### 3.4.5. Calculating vesicle volumes in the dye influx experiments

To quantify the influx rates in the dye influx experiments from the changes in fractional concentration inside a vesicle, we needed to determine the volume of each vesicle. The confocal fluorescence images in the vesicle membrane channel show two-dimensional cross-sections of the vesicles (Supplementary Figure 10), which have a circular shape. The perimeters of these
circles were measured using ImageJ software. The measured radius of a circle, \( w \), was converted to the vesicle’s spherical radius, \( R \), by

\[
R = \frac{(w^2 + z^2)}{2z}
\]

where \( z \) is the height of the focal distance from the coverslip surface, which was set to 8 \( \mu \)m in all dye influx experiments. The measured vesicle volumes changed during the experiments, which were mostly fluctuating throughout time-lapsed imaging while some vesicles showed increasing sizes over time. To account for these measurement variations, for each vesicle, we calculated the mean volume and associated standard errors across all time points. The mean volume and standard errors were then used in obtaining the Gaussian distribution of the influx rate (section 3.4.6).

### 3.4.6. Regression analysis of fractional intensity data

To determine influx rates into each vesicle using Equation 12, we first converted the time-lapse measurements of fractional intensities into piecewise fractional concentrations using the assumption that the measured fluorescence intensity is proportional to the dye concentration.

We performed regression analysis using the diffusion models developed in sections 3.4.1 and 3.4.3 to quantify the four kinetic parameters that described influx kinetics for each vesicle. These parameters are 1) the initial fractional intensity (\( f_0 \)), 2) the linear leaky transport rate (\( k_0 \)), 3) the time at which fast influx starts (\( t_1 \)), and 4) the influx time constant (\( \tau \)). For vesicles that experienced two fast influx events in the nanotube channel dye influx experiment, two additional parameters, the second influx time constant (\( \tau_2 \)) and the time when the second influx starts (\( t_2 \)), were also fit.
The regression was performed by using the nonlinear least-squares solver (“lsqcurvefit”) in MATLAB and the four (or six) parameters. The 95% confidence interval of $\tau$ during the regression were calculated using the “nlparci” function in MATLAB, from which the standard error of $\tau$ for each vesicle was calculated. The standard errors in vesicle volumes ($V$) were also calculated from the measurements of vesicle diameters in the confocal image. The values of the fast influx rate ($k = \frac{V}{\tau}$) and the second fast influx rate ($k_2 = \frac{V}{\tau_2}$) for the corresponding vesicles were then calculated. Finally, the standard errors and 95% confidence intervals of $k$ and $k_2$ were calculated through propagation of error.

To obtain the distribution of influx rates for the vesicles that accounts for the uncertainties in fit, a Gaussian distribution function was fitted to each influx rate using the calculated influx rate and standard error. The probability density function of influx rates of all measured vesicles was obtained by summing the Gaussian distribution of each influx rate in the range of 0 to 120 $\mu m^3/min$ and normalizing the integral to 1. The cumulative distribution function was calculated by numerically integrating the probability density function of the influx rates.
Figure S3.2. Example plots of measured fractional intensities of GUVs and corresponding regression curves. The red curve is the measured fractional intensities of tracked GUVs in the dye influx experiment with DNA nanopores. The blue dash curve is the regression curve by fitting the diffusion model to the measurements (section 3.4.6). The vertical dash-dotted lines in the plots, in the plots for vesicles that experienced fast influx, indicate the time when the fast influx starts ($t_1$), obtained through regression. The plots are ordered by decreasing vesicle volumes, and vesicle numbers are shown inside the plots (number 1 is the plot of the largest vesicle). The influx rates, $k$, and the times at which fast influx of TAMRA starts, $t_1$, are determined through regression. The volumes of the GUVs, $V$, are measured in the confocal images during the dye influx experiment (section 3.4.6) and are also given on the plots. These parameters for the vesicles in the figure are listed in Table S3.4.
Table S3.4. Kinetic parameters for vesicles in Figure S3.2.

<table>
<thead>
<tr>
<th>Vesicle Number</th>
<th>$a \text{ (min}^{-1}\text{)}$</th>
<th>$f_0$</th>
<th>$t_1 \text{ (min)}$</th>
<th>$V \text{ (um}^2\text{)}$</th>
<th>$k_f \text{ (um}^3\text{ min}^{-1}\text{)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.63E-04</td>
<td>0.032</td>
<td>51.10</td>
<td>2.65E+04</td>
<td>18.13</td>
</tr>
<tr>
<td>2</td>
<td>1.26E-04</td>
<td>0.066</td>
<td>71.84</td>
<td>2.53E+04</td>
<td>12.10</td>
</tr>
<tr>
<td>3</td>
<td>2.30E-04</td>
<td>0.058</td>
<td>73.42</td>
<td>2.34E+04</td>
<td>14.29</td>
</tr>
<tr>
<td>4</td>
<td>8.42E-05</td>
<td>0.087</td>
<td>90.00</td>
<td>1.79E+04</td>
<td>9.15</td>
</tr>
<tr>
<td>5</td>
<td>7.95E-05</td>
<td>0.062</td>
<td>65.72</td>
<td>1.69E+04</td>
<td>12.48</td>
</tr>
<tr>
<td>6</td>
<td>4.82E-04</td>
<td>0.068</td>
<td>115.93</td>
<td>1.66E+04</td>
<td>13.09</td>
</tr>
<tr>
<td>7</td>
<td>2.22E-04</td>
<td>0.078</td>
<td>68.39</td>
<td>1.59E+04</td>
<td>12.07</td>
</tr>
<tr>
<td>8</td>
<td>5.43E-04</td>
<td>0.104</td>
<td>36.86</td>
<td>1.55E+04</td>
<td>8.90</td>
</tr>
<tr>
<td>9</td>
<td>2.22E-14</td>
<td>0.082</td>
<td>42.20</td>
<td>1.28E+04</td>
<td>81.82</td>
</tr>
<tr>
<td>10</td>
<td>1.26E-04</td>
<td>0.096</td>
<td>90.00</td>
<td>1.25E+04</td>
<td>46.89</td>
</tr>
<tr>
<td>11</td>
<td>5.85E-04</td>
<td>0.140</td>
<td>104.03</td>
<td>1.20E+04</td>
<td>5.53</td>
</tr>
<tr>
<td>12</td>
<td>1.99E-04</td>
<td>0.081</td>
<td>62.37</td>
<td>1.19E+04</td>
<td>8.62</td>
</tr>
<tr>
<td>13</td>
<td>2.35E-14</td>
<td>0.133</td>
<td>47.65</td>
<td>1.17E+04</td>
<td>6.99</td>
</tr>
<tr>
<td>14</td>
<td>2.22E-14</td>
<td>0.103</td>
<td>33.86</td>
<td>1.17E+04</td>
<td>7.41</td>
</tr>
<tr>
<td>15</td>
<td>4.82E-04</td>
<td>0.256</td>
<td>135.49</td>
<td>1.13E+04</td>
<td>3.65</td>
</tr>
<tr>
<td>16</td>
<td>3.27E-05</td>
<td>0.098</td>
<td>25.02</td>
<td>1.12E+04</td>
<td>2.33</td>
</tr>
<tr>
<td>17</td>
<td>4.23E-04</td>
<td>0.050</td>
<td>119.91</td>
<td>1.11E+04</td>
<td>69.91</td>
</tr>
<tr>
<td>18</td>
<td>4.38E-04</td>
<td>0.096</td>
<td>27.88</td>
<td>1.10E+04</td>
<td>5.85</td>
</tr>
<tr>
<td>19</td>
<td>5.96E-04</td>
<td>0.127</td>
<td>1.00</td>
<td>1.01E+04</td>
<td>0.00</td>
</tr>
<tr>
<td>20</td>
<td>1.73E-04</td>
<td>0.100</td>
<td>63.35</td>
<td>9.99E+03</td>
<td>10.43</td>
</tr>
<tr>
<td>21</td>
<td>1.04E-03</td>
<td>0.089</td>
<td>1.03</td>
<td>9.90E+03</td>
<td>0.00</td>
</tr>
<tr>
<td>22</td>
<td>8.19E-04</td>
<td>0.129</td>
<td>4.58</td>
<td>9.82E+03</td>
<td>0.00</td>
</tr>
<tr>
<td>23</td>
<td>6.38E-04</td>
<td>0.148</td>
<td>1.00</td>
<td>9.36E+03</td>
<td>60.21</td>
</tr>
<tr>
<td>24</td>
<td>1.16E-04</td>
<td>0.103</td>
<td>29.99</td>
<td>9.09E+03</td>
<td>8.32</td>
</tr>
<tr>
<td>25</td>
<td>3.42E-04</td>
<td>0.059</td>
<td>61.90</td>
<td>8.70E+03</td>
<td>6.20</td>
</tr>
<tr>
<td>26</td>
<td>5.85E-05</td>
<td>0.123</td>
<td>65.82</td>
<td>8.55E+03</td>
<td>6.85</td>
</tr>
<tr>
<td>27</td>
<td>2.22E-14</td>
<td>0.113</td>
<td>26.48</td>
<td>8.54E+03</td>
<td>7.10</td>
</tr>
<tr>
<td>28</td>
<td>1.04E-05</td>
<td>0.121</td>
<td>29.19</td>
<td>8.06E+03</td>
<td>4.70</td>
</tr>
<tr>
<td>29</td>
<td>7.40E-04</td>
<td>0.081</td>
<td>78.04</td>
<td>7.69E+03</td>
<td>12.69</td>
</tr>
<tr>
<td>30</td>
<td>4.04E-04</td>
<td>0.113</td>
<td>54.81</td>
<td>7.67E+03</td>
<td>7.04</td>
</tr>
<tr>
<td>31</td>
<td>1.47E-04</td>
<td>0.088</td>
<td>71.24</td>
<td>7.55E+03</td>
<td>5.62</td>
</tr>
<tr>
<td>32</td>
<td>1.25E-03</td>
<td>0.114</td>
<td>83.79</td>
<td>7.55E+03</td>
<td>87.62</td>
</tr>
<tr>
<td>33</td>
<td>4.77E-04</td>
<td>0.079</td>
<td>65.14</td>
<td>7.43E+03</td>
<td>5.90</td>
</tr>
<tr>
<td>34</td>
<td>8.64E-05</td>
<td>0.121</td>
<td>74.79</td>
<td>7.36E+03</td>
<td>3.38</td>
</tr>
<tr>
<td>35</td>
<td>8.14E-05</td>
<td>0.123</td>
<td>57.13</td>
<td>7.13E+03</td>
<td>2.77</td>
</tr>
</tbody>
</table>
Figure S3.3. The probability density distributions of the influx rates in the nanopore and capped nanopore dye influx experiments. The influx rates were determined by fitting the diffusion model (Equation 12) to the measured influx kinetics of GUVs tracked in the experiments in which DNA pores (N = 171) and capped DNA pores (N = 87) were added to vesicles respectively. The influx rates for each GUV were then fit to Gaussian distributions (section 3.4.6).
Figure S3.4. Example plots of measured and fitted fractional intensities of GUVs in experiments where DNA nanochannels are added to GUVs. The red curve in each plot is the measured fractional intensity of a tracked GUV. The blue dash curve is the regression curve produced by fitting the diffusion model to the measurements (Equation 14 or 15). The vertical dash-dotted lines, in the plots for vesicles that experienced fast influx, indicate the time when the fast influx starts ($t_1$), obtained through regression. The influx rates, $k$, and the times at which fast influx of TAMRA starts, $t_0$, are determined through regression. The volumes of the GUVs, $V$, are measured in the confocal images during the dye influx experiment (section 3.4.6). These parameters for the vesicles in the figure are listed in Table S3.5.
Table S3.5. Kinetic parameters for vesicles in Figure S3.4.

<table>
<thead>
<tr>
<th>Vesicle Number</th>
<th>$a$ (min$^{-1}$)</th>
<th>$f_0$</th>
<th>$t_1$ (min)</th>
<th>$V$ (μm$^3$)</th>
<th>$k_F$ (μm$^3$ min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.57E-04</td>
<td>0.074</td>
<td>156</td>
<td>4.09E+05</td>
<td>8.53</td>
</tr>
<tr>
<td>2</td>
<td>4.41E-04</td>
<td>0.076</td>
<td>94</td>
<td>1.33E+05</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>5.74E-06</td>
<td>0.084</td>
<td>1</td>
<td>1.14E+05</td>
<td>54.34</td>
</tr>
<tr>
<td>4</td>
<td>4.45E-04</td>
<td>0.084</td>
<td>136</td>
<td>7.78E+04</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>3.03E-04</td>
<td>0.054</td>
<td>367</td>
<td>7.14E+04</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>2.38E-04</td>
<td>0.069</td>
<td>447</td>
<td>5.81E+04</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>4.71E-04</td>
<td>0.099</td>
<td>173</td>
<td>5.02E+04</td>
<td>1.52</td>
</tr>
<tr>
<td>8</td>
<td>2.22E-14</td>
<td>0.115</td>
<td>40</td>
<td>4.93E+04</td>
<td>38.50</td>
</tr>
<tr>
<td>9</td>
<td>2.87E-04</td>
<td>0.075</td>
<td>258</td>
<td>4.92E+04</td>
<td>2.48</td>
</tr>
<tr>
<td>10</td>
<td>2.89E-14</td>
<td>0.098</td>
<td>5</td>
<td>3.73E+04</td>
<td>23.15</td>
</tr>
<tr>
<td>11</td>
<td>1.79E-04</td>
<td>0.066</td>
<td>314</td>
<td>3.59E+04</td>
<td>1.43</td>
</tr>
<tr>
<td>12</td>
<td>2.18E-04</td>
<td>0.080</td>
<td>439</td>
<td>3.37E+04</td>
<td>24.90</td>
</tr>
<tr>
<td>13</td>
<td>4.14E-04</td>
<td>0.110</td>
<td>219</td>
<td>3.17E+04</td>
<td>0.39</td>
</tr>
<tr>
<td>14</td>
<td>5.34E-04</td>
<td>0.123</td>
<td>62</td>
<td>3.15E+04</td>
<td>0.01</td>
</tr>
<tr>
<td>15</td>
<td>2.44E-04</td>
<td>0.097</td>
<td>224</td>
<td>2.19E+04</td>
<td>3.29</td>
</tr>
<tr>
<td>16</td>
<td>2.26E-04</td>
<td>0.059</td>
<td>24</td>
<td>1.87E+04</td>
<td>5.26</td>
</tr>
<tr>
<td>17</td>
<td>6.14E-04</td>
<td>0.118</td>
<td>2</td>
<td>1.83E+04</td>
<td>0.00</td>
</tr>
<tr>
<td>18</td>
<td>4.74E-04</td>
<td>0.117</td>
<td>147</td>
<td>1.61E+04</td>
<td>1.12</td>
</tr>
<tr>
<td>19</td>
<td>1.09E-06</td>
<td>0.140</td>
<td>3</td>
<td>1.60E+04</td>
<td>10.92</td>
</tr>
<tr>
<td>20</td>
<td>2.60E-04</td>
<td>0.099</td>
<td>447</td>
<td>1.59E+04</td>
<td>0.00</td>
</tr>
<tr>
<td>21</td>
<td>3.32E-04</td>
<td>0.049</td>
<td>386</td>
<td>1.57E+04</td>
<td>1.31</td>
</tr>
<tr>
<td>22</td>
<td>4.17E-04</td>
<td>0.045</td>
<td>225</td>
<td>1.55E+04</td>
<td>26.14</td>
</tr>
<tr>
<td>23</td>
<td>3.65E-04</td>
<td>0.053</td>
<td>243</td>
<td>1.50E+04</td>
<td>19.94</td>
</tr>
<tr>
<td>24</td>
<td>2.22E-14</td>
<td>0.084</td>
<td>23</td>
<td>1.31E+04</td>
<td>6.56</td>
</tr>
<tr>
<td>25</td>
<td>4.07E-04</td>
<td>0.056</td>
<td>1</td>
<td>1.21E+04</td>
<td>0.00</td>
</tr>
<tr>
<td>26</td>
<td>4.37E-04</td>
<td>0.129</td>
<td>167</td>
<td>1.18E+04</td>
<td>0.07</td>
</tr>
<tr>
<td>27</td>
<td>6.54E-04</td>
<td>0.130</td>
<td>2</td>
<td>1.10E+04</td>
<td>0.00</td>
</tr>
<tr>
<td>28</td>
<td>3.35E-04</td>
<td>0.059</td>
<td>99</td>
<td>1.03E+04</td>
<td>1.08</td>
</tr>
<tr>
<td>29</td>
<td>5.17E-04</td>
<td>0.120</td>
<td>21</td>
<td>9.07E+03</td>
<td>0.00</td>
</tr>
<tr>
<td>30</td>
<td>2.22E-14</td>
<td>0.092</td>
<td>40</td>
<td>8.40E+03</td>
<td>31.75</td>
</tr>
<tr>
<td>31</td>
<td>4.98E-04</td>
<td>0.060</td>
<td>202</td>
<td>7.71E+03</td>
<td>3.97</td>
</tr>
<tr>
<td>32</td>
<td>2.22E-14</td>
<td>0.168</td>
<td>129</td>
<td>7.42E+03</td>
<td>45.08</td>
</tr>
<tr>
<td>33</td>
<td>5.23E-04</td>
<td>0.151</td>
<td>1</td>
<td>7.37E+03</td>
<td>0.00</td>
</tr>
<tr>
<td>34</td>
<td>6.19E-04</td>
<td>0.140</td>
<td>4</td>
<td>7.36E+03</td>
<td>0.00</td>
</tr>
<tr>
<td>35</td>
<td>2.22E-14</td>
<td>0.137</td>
<td>18</td>
<td>6.49E+03</td>
<td>6.89</td>
</tr>
</tbody>
</table>
3.4.7 Theoretical rate of TAMRA transport through a single DNA seed pore

We hypothesize that the rate of one-dimensional transport of TAMRA within the DNA pore that spans across the vesicle membrane follows Fick’s law of diffusion without significant effects due to transport along the pore surface. In this case, the net flux of TAMRA transport through a single DNA pore is described by Equation 2. Because TAMRA concentration inside the vesicle ($C_{in}$) increases over time during the influx event, we define the influx rate

$$k = \tau \cdot V = \frac{AD}{L}$$

(18)

as a measurement of how fast TAMRA transports through the pores. Here, $\tau$ is the time constant of the negative exponential kinetics, $V$ is the volume of the vesicle, $A$ is the cross-sectional area of the pore, $L$ is the length of the pore, and $D$ is the diffusion coefficient of TAMRA.

To calculate the theoretical influx rate for a single pore, we calculated the cross-sectional area to be $A = 42 \pm 3.4$ nm$^2$ after approximating the cross-section as a circle with a measured diameter of $7.3 \pm 0.4$ nm (Supplementary Fig. 2), and the length of the pore was measured $L = 61.1 \pm 2.1$ nm.

In the dye influx experiments, TAMRA was in a 0.2 M glucose solution at 37°C. The diffusion coefficient of TAMRA in such conditions can be calculated from the diffusion
The coefficient of TAMRA, $D_1=2.8 \pm 0.3 \times 10^{-6} \text{cm}^2/\text{s}$, measured in 10% glycerol at room temperature\textsuperscript{137} based on the Stoke-Einstein Equation, which predicts

$$\frac{D_1}{D_2} = \frac{T_1 \mu_2}{T_2 \mu_1}$$

which accounts for the diffusion coefficient’s dependences on temperature and viscosity. Here, $D_1$ and $D_2$ are the diffusion coefficients in the two conditions. $T_1$ and $T_2$ are the corresponding absolute temperatures. $\mu_1$ and $\mu_2$ are the corresponding dynamic viscosities. The dynamic viscosity of 10% glycerol at 37°C is $\mu_1=9.30 \times 10^{-4} \text{Pa} \cdot \text{s}$\textsuperscript{101} The dynamic viscosity of 0.2 M glucose solution at 37°C is $\mu_2=7.28 \times 10^{-4} \text{Pa} \cdot \text{s}$ after interpolation\textsuperscript{101} The diffusion coefficient of TAMRA in the experimental conditions is then calculated to be $D_2=3.58 \pm 0.4 \times 10^{-6} \text{cm}^2/\text{s}$. Using this value, we find the theoretical influx rate of a single DNA pore is $k = 14.7 \pm 1.8 \mu\text{m}^3/\text{min}$. 

![Graph of Amplitude vs Frequency](image-url)
Figure S3.5. Fourier transform of the probability density distribution of influx rates of TAMRA into DNA seed pores. The discrete probability density function of influx rates \( (k) \) in between 15 and 120 \( \mu m^3/min \) was normalized by subtracting the mean value (removing the DC bias) and removing the linear trends using the “detrend” function in MATLAB before taking the Fourier transform. The frequency peak at 0.0761 min/\( \mu m^3 \) represents a dominant periodic frequency and corresponds to a dominant period of 13.1 \( \mu m^3/min \) in the probability density function of influx rates.

Figure S3.6. Schematic of the dye influx experiment setup. DNA nanochannels (blue) are added to a solution containing TAMRA (red) and GUVs (green) on a glass-bottom dish.

3.4.8. Alternative protocols for synthesizing GUVs
Besides the GUV preparation protocol described in Methods, several different GUV preparation methods were tested and the GUV yields were assessed using optical microscopy.

We first adopted the gentle hydration method for GUV preparation\textsuperscript{137}. 400 μl solution containing 330 mM sucrose and TAE buffer was used to hydrate the dried lipid film of 10 mg/ml POPC initially dissolved in chloroform. 50 μl GUV solution was transferred to a glass coverslip to be imaged under bright field (Fig. S3.7).

![Figure S3.7. Bright field microscopy image of GUVs formed from POPC lipids. Most vesicles are multilamellar. Scale bar, 5 μm.](image)

We then adapted the gel-assisted hydration method reported by Horger et al\textsuperscript{116} (see Methods). First, a 5 mg/ml lipid mix consisting of 83 wt% DPhPC, 15 wt% biotinyl PC, and 2 wt% TopFluor PC. The dried hybrid films of agarose gel and lipids were hydrated with 500 mM
potassium chloride. Most GUVs formed using this protocol were unilamellar but the yield was relatively low (Fig. S3.8).

Figure S3.8. Confocal fluorescence image of GUV formed using gel-assisted hydration method. Different lipid composition and hydration solution from those described in Methods was used. The yield of GUVs were low (one of two GUVs per field of view when randomly searching on the microscope).

3.4.9 DNA nanopore stability in low-magnesium conditions

The presence of divalent cations contributes to the deformation and ruptures of lipid vesicles. Adding GUVs (prepared as described in Method) to a 12.5 mM magnesium acetate solution caused all vesicles to rupture. However, DNA origami structures, including the designed DNA nanopore, requires magnesium ions to maintain their scaffolded structures. However, the specific magnesium ion concentration requirement would vary for the DNA origami
geometries. Thus, we used TEM imaging to assess DNA nanopore stability in different ionic conditions.

We first tested whether DNA nanopores were stable in 500 mM monovalent cations in the absence of divalent cations. Specifically, DNA nanopores were formed as described in 2.4.3 and then filter purified in TAE buffer supplemented with 500 mM potassium chloride. The nanopores were then incubated at room temperature for an hour before being deposited onto a TEM grid. The TEM image showed the nanopores were completely deformed (Fig. S3.9).

**Figure S3.9.** TEM image of DNA nanopores that were incubated in TAE buffer supplemented with 500 mM potassium chloride for an hour at room temperature.
After confirming the GUVs remained stable in 3 mM magnesium acetate by taking bright field microscopic image (Fig. S3.10), we tested whether DNA nanopores retained their structures in 3 mM magnesium. The formed nanopores were filter purified in TAE buffer supplemented with 3 mM magnesium acetate and then incubated at room temperature for an hour. The TEM image of the nanopores after the incubation showed that the nanopores retained their structures.

**Figure S3.10.** Bright field image of GUVs in 3 mM magnesium acetate. Imaged on a glass-bottom dish. Most GUVs remained intact. Scale bar, 5 μm.
**Figure S3.11.** TEM image of DNA nanopores that were incubated in TAE buffer supplemented with 3 mM magnesium acetate. Imaged after 1-hour incubation at room temperature.

### 3.4.9 DNA nanochannels with a PEG coating

In chapter 2, we developed DNA nanotubes with a PEG coating, where the coating was used to reduce the undesired interaction between the nanotubes and glass surface in the presence of serum. The same PEG coating can be applied to DNA nanochannels as a proposed approach to minimize unspecific nanochannel interactions with GUV membranes. This is a preventive measure in case there was a strong unspecific adsorption of DNA to GUV membranes. Such adsorption was not observed under confocal microscopy and therefore the PEG coating was not used in the dye influx experiments.
DNA nanochannels with a PEG coating assembled on DNA nanopores were assembled using DNA tiles conjugated to PEG-20kDa with the same method as described in 5.4.1. The PEF-coated DNA nanochannels were further concentrated through spin filtration and then modified with cholesterol. After mixing the GUVs and cholesterol modified nanochannels in the same way as in dye influx experiments (see Methods), we observed the mixture using confocal microscopy (Fig. S3.12). The image showed many nanochannels incorporated onto GUVs through the interactions between the nanopores attached to the nanochannels and vesicle membranes. No unspecific adsorption of nanochannels to vesicle membranes were observed.

**Figure S3.12.** Confocal fluorescence image of PEG-coating nanochannels and GUVs. **a.** Multicolor image created by merging a DNA nanochannel (red) image and a GUV (green) image. **b.** Fluorescence image of the DNA nanopores attached to the nanochannels captured at the same location.

### 3.4.10 Concentrate DNA nanochannels through dialysis
Concentrating DNA nanotubes or nanochannels through spin filtrations using centrifugal units (Amicon UFC510024) resulted in significant length shortening due to the shear stress during centrifugation. An alternative approach was developed to concentrate formed DNA nanochannels based on dialysis using Slide-A-Lyzer™ MINI Dialysis Device with 3.5kDa molecular weight cutoff (Catalog # 88400, ThermoFisher Scientific, Waltham, MA, USA). 400 μl DNA nanochannels were assembled following the protocol in 2.4.3 and transferred into the inner filter unit. 15 ml 6 mM PEG-5kDa was dissolved in TAEM buffer and was transferred into the outer filter unit. The assembled inner and outer unit was left on a shaker for 28 hours, during which the higher osmotic pressure in the outer unit led to water diffusion from the inner solution of DNA nanochannels. The DNA nanochannels before the dialysis and after the dialysis were imaged on glass coverslips with fluorescence microscopy to assess concentrating efficiencies.

Figure S3.13. Fluorescence images of the same volumes of DNA nanochannels before dialysis and after dialysis. The concentration of the nanochannels were significantly higher after the dialysis without apparent length reduction. Scale bar, 5 μm.
Chapter 4: DNA Nanopore-based Biosensor for Nucleic Acid Detection

4.1 Introduction

Biosensors developed through the engineering of biological processes, including enzyme reactions, antibody and DNA bindings, and cell organelles, have become an attractive field of study. Many recent implementations are shown to address the unmet needs in medical diagnostics, drug discovery, food safety, and environmental monitoring. Among these applications, the advances in nucleic acid diagnostics are particularly exciting as they have enabled development of low-cost biosensors for detecting pathogens and human health biomarkers, which would vastly improve the access to health care. Since the inception of the biosensors in the 1960s, various biosensing approaches being used are enzyme-based, tissue-based, immunosensors, and DNA-based.

Synthetic biology has largely propelled the development of biosensors in the past several years. Many biosensors based on synthetic biology use living cells, leading to the development of cell biosensors. Taking advantages of various metabolic reactions using intracellular enzymes, cell biosensors benefit from long service life and high reproducibility. However, cell biosensors have dependence on their own physiological conditions and therefore are not suitable for uses for cytotoxic or many environmental samples. To address these limitations, efforts to engineer cell-free expression systems as point-of-use sensing platforms have gained much success. The cell-free systems use extracted components from living cells that consist of DNA templates, transcriptional and translational enzymes, and necessary substrates. The design principle aims at
converting analyte recognition to the expression of reporter gene. The main advantages of cell-free systems include toxicity tolerance, high sensitivity (detection limit on nanomolar level), and stability upon lyophilization. So far, the cell-free biosensors have demonstrated detection of metal ions, antibiotics, and bacteria and viruses. However, the cell-free systems contain energy sources, enzymes, and cofactors to support gene expression, which can be prone to undesired interactions with different biological samples introduced.

As an alternative, DNA nanostructure-based biosensors emerged as a platform for detection of diseases. DNA biosensors utilize the property that single-stranded nucleic acid can recognize and bind to its complementary nucleic strand in the samples, making them particularly useful in nucleic acid diagnostics. Leveraging the specificity in DNA interactions and simplicity in design, different types of DNA nanobiosensors that transduce the molecular signals of target DNA and RNA have been reported, such as DNA origami, DNA tetrahedron, and DNA-labeled quantum dots. Among various geometries of DNA nanobiosensors, DNA nanopores, which mimic proteins pores and can insert into lipid bilayers, show great potentials in label-free sensing of nucleic acids. The nucleic acid detection with DNA nanopores was initially made through nanopore conductance monitoring for translocation events, by applying a voltage across a plane lipid bilayer membrane on which single nanopores inserted onto and monitoring the changes in the current across the membrane. However, while this technique enables ultra-sensitive single molecule detection, it requires finely tuned electronic equipment and therefore not suitable to be used as point-of-use devices.

Recently, DNA origami nanopores that can switch to an open or closed state upon specifically capturing nucleic acid targets at pre-designed sites have been reported. Since the molecular transport through the nanopores is controlled by the opening and closing of the
nanopores, the recognition of the target nucleic acids can be reported as molecular fluxes across the membranes that nanopores are inserted in. Thus, if the molecular fluxes are to be easily measured with a simple device, the DNA nanopores can serve as biosensors to detect the presence of specific nucleic acid sequences.

Furthermore, since a series of technological advances has been made in developing DNA aptamers with high levels of target-binding affinity, such DNA aptamers can be potentially integrated into a DNA nanopore-based biosensing system for detection of various biomolecular species. The aptamers can act as transducers for converting protein, peptide, metal ion, or other biomolecule inputs into the DNA species that activate the transport through DNA nanopores. Such a nanopore-based biosensor that can provide in situ detection of the presence and relative abundance of proteins would be particularly interesting, since the current assays, including immunosorbent assays, mass spectrometry, and western blot, mostly require sample processing where the proteins cannot be recovered.

Human α-thrombin is a multifunctional serine protease and is important in thrombosis, homeostasis, and inflammation. Thus, thrombin-binding aptamers are of particular interests in medical and clinical field. Agrawal and Schulman developed a reaction process, in which a thrombin-binding DNA aptamer interacted with thrombin proteins reported the thrombin concentration as the concentration of an output DNA oligonucleotide strand. Based on the signal exchange reaction that based on DNA strand displacement reactions reported in this study, we can redesign the reactions to convert thrombin inputs to a DNA strand that switches DNA nanopores to an open state. In this way, the presence and concentrations of thrombin proteins can be transduced to the nanopore openings and then molecular fluxes across the membrane the
nanopores are inserted in. Therefore, measurements of the molecular fluxes would enable the
detection of thrombin.

Here, we designed a biosensing system consisted of DNA origami nanopores and giant
unilamellar vesicles (GUVs) that translates specific DNA sequence to a glucose signal, which is
then readily monitored with a glucose meter. Specifically, we exploited a DNA origami
nanopore we previously designed that was capable of inserting onto GUV membranes and
inducing diffusive transport of small molecules across membranes. When bound to a DNA
origami cap that could plug the molecular transport, however, the nanopores remained in a
closed state. We modified the binding interface between the DNA nanopore and the cap to
present six sites to capture the target single-stranded DNA species. The capture would trigger a
strand displacement reaction to detach the cap from the nanopore for a close-to-open
transformation. We demonstrated that the glucose efflux through the opened nanopores led to
increases in bulk glucose concentration, which could be easily measured with a commercial
glucose meter. Remarkably, positive responses were triggered in the presence of 20 nM target
DNA in as soon as 15 minutes during 37 °C incubation. Our system shows simplicity in use as it
only requires a one-pot mixing and a short isothermal incubation to report the detection.
Therefore, this work presents a proof-of-concept DNA-based biosensor for rapid nucleic acid
detection, providing a new approach for developing point-of-care diagnostics devices. Moreover,
we redesign the nanopore-based biosensor system that incorporate a thrombin-binding aptamer
that would trigger nanopore opening and glucose efflux in the presence of thrombin proteins.
Although further experiments are needed to confirm and characterize the thrombin detection
capacities, we showed our nanopore-based biosensor had potentials to be extended to be in situ
protein sensors.
4.2 Methods

Materials

All DNA oligodeoxynucleotides were purchased from Integrated DNA Technologies (IDT, Coralville, Iowa, USA) with HPLC purification, except the standard desalting for the DNA nanopore and DNA cap staple strands. The 7,240bp M13mp18 scaffold strand purchased from Bayou Biolabs (Los Angeles, CA, USA) Tris/acetate/EDTA (TAE) buffer and PBS buffer was purchased from ThermoFisher Scientific (Waltham, MA, USA). All lipids were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Dextrose (glucose) was purchased from Millipore Sigma (St. Louis, MO, USA). Human α-thrombin was purchased from Haematologic Technologies, Inc. (Essex, VT) and dissolved in 50% H2O/glycerol.

DNA Nanopore and DNA Cap Assembly

A 400 μl mixture containing 5 nM scaffold strand, 200 nM corresponding staple strand mix, 100 nM corresponding adapter strands, and 25 nM attachment strand mix, was first prepared in TAE Mg2+ buffer (40 mM Tris-Acetate, 1 mM EDTA) with 12.5 mM magnesium acetate added (TAEM). The assembly mixture was subjected to a thermal annealing ramp with an Eppendorf Mastercycler. The assembled nanopores or caps were then purified to removed excessive DNA strands by using incubation buffer and a 100k MWCO Amicon Ultra centrifugal filter device (Millipore Sigma, UFC510096). For DNA nanopores, 0.5 μl of 100μM ATTO647-modified DNA strand was added to the 20 μl purified sample, and for DNA caps, ATTO488-modified DNA strand was added to the 20 μl purified sample. The sample was incubated at room temperature for 15 min. The concentration of fluorescently labeled nanopores was approximately 26 nM, and the DNA cap concentration was approximately 38 nM, determined by counting the
number of pores per field of view (86 μm × 86 μm) adsorbed to a glass slide from a specific reaction volume in fluorescence micrographs captured using a fluorescence microscope (Olympus IX71) with a 60×/1.45 NA oil immersion objective lens and a 1.6x magnifier lens. 15 μl nanopores and 20 μl caps were mixed and kept in 4°C overnight for the nanopores and caps to bind. To modify the capped DNA nanopores with cholesterol, 2 μl DNA-cholesterol conjugate at 10 μM was incubated at 50 °C for 10 minutes to alleviate cholesterol aggregation before being added to 35 μl capped DNA nanopores. The solution was then incubated for 10 minutes at room temperature.

**Fluorescence Microscopy Measurements of DNA Nanopore Uncapping Efficacies**

The sample of capped DNA nanopores were diluted 800 times by mixing 0.2 μl capped nanopores and 159.8 μl incubation buffer. 6 μl of diluted sample was deposited onto an 18 mm rectangular glass coverslip, which was then placed onto a glass slide. The slide was imaged on an inverted microscope (Olympus IX71) using a 60x/1.45 NA oil immersion objective lens and a 1.6x magnifier lens. Grayscale filtered images (ATTO488 and ATTO647) were taken for three random locations on the slide. The grayscale images at each location were merged to create a composite two-color image that showed DNA nanopores and DNA caps in red and green, respectively. A MATLAB script was developed to quantify the fraction of DNA nanopores bound to DNA caps in each image. A nanopore was considered capped if its center-of-mass position was within proximity (<5 pixels, i.e., <0.8 μm) with that of a DNA cap.

**Wash GUVs with Incubation Buffer**
200 µl GUVs prepared in 100 mM glucose solution was transferred to a 0.6 ml centrifugal tube and 400 µl incubation buffer was added. The vesicles in the tube were centrifuged at 3,000 g for 5 minutes, after which separation of GUVs from the supernatant could be observed. After removing 400 µl supernatant, 400 µl incubation buffer was added to the tube. The tube is centrifuged at 3,000 g for 3 minutes, followed by removing the entire supernatant from the vesicle pellets at the bottom of the tube and adding 600 µl incubation buffer. This step was repeated one more time. To completely wash away the glucose solution in the vesicle pellet, this washing step consisted of centrifugation, supernatant removal, and buffer addition was repeated 4 more times with an additional procedure that the pellet was gently resuspended with a transfer pipette after adding the buffer into the tube. To measure the glucose concentration of the vesicle solution after washing, after removing the supernatant the last time, 70 µl incubation buffer was added to the tube instead of 600 µl and the pellets were resuspended. The tube was centrifuged at 2,000 g for 2 min. 0.65 µl supernatant was transferred to a glucose strip to measure glucose concentration and then the supernatant is removed. If the measured glucose concentration is above the detection limit of the glucose meter (10 mg/ml), repeat the washing step one more time until the reading is below the detection limit.

**Glucose Release Assay**

70 µl incubation buffer was added to the vesicle pellets after washing for a total volume of approximately 100 µl. The vesicle pellets were resuspended with a transfer pipette. The vesicle solution was then transferred into 0.2 ml PCR tubes with 17.75 µl solution in each tube. For the experiments with capped DNA nanopores, 6.25 µl capped DNA pores along with 1 µl either incubation buffer or specified concentration of uncapping strand was added to the tubes.
For control experiments without capped DNA nanopores, 6.25 µl incubation buffer along with 1 µl either specified amount unmatching strand or incubation buffer were added to the tubes. The tube was then incubated in a benchtop incubator set at 37°C. The GUVs would gradually sink onto the bottom of the tube due to density gradients between inner solution and outer solution. To avoid taking out vesicles during measurements, 0.65 µl at the surface of the solution in the tube was transferred onto a glucose meter test strip for glucose concentration measurements at different time points. After the measurement at the last time point, the samples in tubes were sonicated for 45 minutes at 60 °C incubation in an ultrasonic cleaner (Branson Ultrasonic Cleaner 1510R-DTH) to break all the vesicles so that all encapsulated glucose was release. 0.65 µl of the solution in the tube was then transferred to a test strip to measure glucose concentration.

**Fluorescence assay for measuring DNA outputs to thrombin inputs**

To determining how concentrations of the DNA key change as different concentrations of thrombin are added to the aptamer and transduction system, we prepared 50 nM aptamer components (W, X, Y, O, and Z strands) and used a fluorescence assay to measure reaction kinetics. The O strand was labeled with an Iowa black FQ quencher at 3’ end and Z strand was labeled with FAM fluorophore at 5’ end. Firstly, 20 µM of XY and OZ strands were pre-annealed in PBSM buffer (38.4 mM NaCl, 0.8 mM KCl, 2.8 mM Na₂HPO₄, 0.5 mM KH₂PO₄, 3.1 mM Mg(C₂H₃O₂)₂) using the annealing protocol (see 2.4.3). Different concentrations of thrombin (0, 25, 50, 75, 100, 150, and 200 nM) thrombin was then mixed with 100 nM aptamer components (W, XY, OZ) for a total volume of 100 µl for each mixture. The mixtures were made in different wells of a 96-well qPCR plate (Agilent, Santa Clara, CA, USA). The plate was
then measured in a plate reader (Synergy H1) for fluorescence intensities in the FAM channel every 30 seconds under 37°C incubation.

4.3 Results and Discussion

Program the opening of DNA nanopores

To develop a DNA nanopore system in which the pores were initially closed but could be switched to an open state, we redesigned the binding interface between a DNA nanopore and a cap to be responsive to nucleic acid signals. In chapter 3, we showed the 12 overhangs for the attachment of 12 DNA-cholesterol conjugates to DNA nanopore facilitated nanopore insertions into lipid bilayer membranes. The cholesterol modified nanopores could spontaneously insert onto GUVs and induce diffusive transport of small molecules across the membranes. We also designed a DNA cap that had a very narrow neck, which could bind to the open end of the nanopore through sticky end hybridizations. The transport through the nanopore was stopped when caps were bound.
**Figure 4.1.** Capping and uncapping of DNA nanopores. 

(a) Design of the adapters on the nanopore and the cap. The complementary sticky ends on the adapters enable the binding of the nanopore and the cap. The additional 5’ single-stranded region (red) on the sticky-end strands facilitates toehold-mediated strand displacement that removes the sticky-end strands and causes the cap detachment. 

(b) Multicolor fluorescence images of DNA nanopores (red) bound and caps (green). Left: Nanopores are bound to caps in the absence of DNA key. Right: caps detach from the caps after adding 1 μM DNA key. Scale bars, 2 μm. 

(c) Fractions of nanopores bound to caps after 30-minute incubation of 2.5 nM capped nanopores with different concentrations of DNA key. The fractions were calculated by dividing the counts of colocalizations of the nanopores and caps by the total number of nanopores in fluorescence images. For DNA concentration of 0 nM, 20 nM, 50 nM, 250 nM, and 1 μM, \( N = 594, 158, 211, 207, 258, 170 \), respectively. Error bars of 95% confidence intervals of the mean.
The bindings of the nanopore and the cap relied on the interactions between the adapters on their ends. Briefly, the 12 sticky ends presented on the nanopore adapter could hybridize with the 12 complementary sticky ends on the cap adapter. Since the sticky-end strands were responsible for the binding, removal of these strands would cause the cap to detach from the nanopore. Therefore, we adapted a terminus activation scheme\textsuperscript{131,151,152} that would allow the sticky-end strands on the cap adapter to be removed through a strand displacement reaction after the capping of nanopores (Fig. 4.1a). Specifically, each of the six sticky-end strands on the cap adapter was modified to present a 7-nt toehold at the 5’ end and to have the same sequence. A DNA key strand fully complementary to the sticky-end strands could first bind to the toeholds and then displace the sticky-end strands, which would remove the sticky-end strands from the interface and thereby detach the caps.

We quantified the detachments of the caps from the nanopores with fluorescence microscopy. DNA nanopore and caps were labeled with two different fluorophores. One-fold excess of DNA cap was mixed with the nanopores and the mixture was incubated at 4 °C overnight. By analyzing the colocalizations of the nanopores and caps in fluorescence images (Fig. 4.1b, left) showed 91% ± 2% nanopores were bound to caps. To test whether DNA key could uncap the nanopores, a final concentration of 1 μl DNA key was then added to 2.5 nM capped nanopores. After a 30-minute incubation at 37°C, the fluorescence images showed only 19% ± 3% nanopores remained capped (Fig. 4.1b, right). We then repeated the experiments to measure the uncapping yields at lower DNA key concentrations. As expected, the measured fraction of nanopores that remained capped (Fig. 4.1c) increased as DNA key concentration decreased. As low as 20 nM DNA key could result in uncapping of 30% nanopores (70% ± 3% capped nanopores).
Characterize DNA nanopore-mediated glucose efflux

We first developed a protocol for preparing giant unilamellar vesicles (GUVs) that encapsulate glucose molecules. Specifically, GUVs that contained fluorescent phospholipids were formed through a polymer-assisted hydration method using 100 mM glucose as the hydration solution. The formation of the GUVs were verified with confocal microscopy (Fig. 4.2a). To remove the glucose outside the GUVs after the GUVs formed, the vesicle solution was subsequently washed with a magnesium-supplemented diluted PBS buffer (denoted incubation buffer) through a centrifugation process (see Methods). The glucose concentration outside the vesicles were below the detection limit of the glucose meter (10 mg/dL) after washing. We then verified the GUVs remained intact after the washing process by adding TAMRA dyes to the glucose solution during the GUV preparation. The GUVs were then imaged under a confocal microscope after washing (Fig. 4.2b), which showed the dyes in the outer solution were removed after centrifugation with no alterations to vesicle morphologies.

As the DNA nanopores have been shown to transport small molecules across lipid membranes, we hypothesized that the cholesterol modified DNA nanopores could induce the efflux of glucose encapsulated in the GUVs. The glucose released would then lead to increasing glucose concentration in the bulk solution, which could be measured as increases in the glucose concentration in the bulk solution (Fig. 2c). However, if capped nanopores were added to the GUVs instead of open nanopores, we should observe no changes in the glucose concentration (Fig. 2d).
Figure 4.2. Encapsulation and release of glucose molecules from GUVs. **a.** Fluorescence image of GUVs prepared in 100 mM glucose solution. **b.** Multicolor fluorescence image showing GUVs (green) encapsulating 100 mM glucose and 1 μM TAMRA dyes (red) remain intact after being washed with magnesium-supplemented PBS buffer. **c.** Schematics showing DNA nanopores mediate efflux of glucose molecules in a GUV. **d.** Schematics showing no glucose efflux occurs when capped nanopores are used. **e.** Schematics of the glucose release experiment with either open or capped nanopores. **f.** Fractions of glucose released from GUVs measured when open nanopores, capped nanopores, and buffer only in the glucose release experiments, and the fractions are averages of $N = 4$, $4$, and $5$ replicates, respectively. Error bars are standard errors of the mean. Scale bars, 10 μm.

To test this hypothesis, we sought to measure the kinetics of the glucose release from the GUVs in the presence of open nanopores and capped nanopores. We first prepared a 25 ul mixture containing 2.5 nM DNA nanopores with cholesterol modifications and resuspended GUVs, and the sample was incubated at 37 °C (Fig. 4.3e). The GUVs sank down to the bottom of
the tube within the first 15 minutes due to the density difference between the vesicle inner solution and the outer bulk solution. The glucose concentration of the bulk solution was measured at $t = 1, 2, 3,$ and 8 hours by transferring 0.65 ul from the top of the solution to a glucose meter test strip.

Because the yield of GUVs varied during the GUV preparation, the amount of glucose encapsulated in the vesicles would vary in each experiment. To calibrate the measure time-lapsed glucose concentrations, at $t = 8$ hours, we measured the total amount of glucose in the sample after breaking GUVs with sonication. The fraction of glucose release was then calculated by dividing the measured glucose concentration at each time point by the total amount of glucose (Fig. 4.2f). We repeated the glucose release experiment but added capped nanopores instead of open nanopores, in addition to a control experiment where only buffer was added to the GUV solution. The non-negligible glucose release in the control experiment showed a baseline glucose efflux rate that is not nanopore-mediated, which was likely due to a small membrane permeability of glucose across. The faster release within the first hour was possibly due to that a fraction of synthetic GUVs had inherent defects that resulted in higher permeabilities. The fractions of glucose released in the presence of capped nanopores were only very close to those in the control experiment in the first 3 hours of incubation, suggesting the capped nanopores did not induce the efflux of glucose. When DNA nanopores were used, however, the distinctly higher fraction of glucose released as early as at 1 hour suggested that the glucose transported across membranes through the open nanopores.

**Fast detection of DNA using a glucose meter**
Now we have demonstrated that the open nanopores, but not capped nanopores, triggered glucose release from GUVs and that the cap bound to the nanopore could be detached using a DNA key strand. We then asked whether this GUV-nanopore system could be used to detect the presence of target DNA (Fig. 4.3a). We hypothesized that after mixing the capped DNA nanopores with cholesterol modification and the GUVs encapsulating glucose, the kinetics of glucose release would remain very slow. However, if the DNA key species was present, the caps on the inserted nanopores were released and the glucose efflux could be detected with a glucose meter (Fig. 4.3b).
Figure 4.3. Capped DNA nanopores and GUVs encapsulating glucose can be used for fast detection of target DNA species. a. Schematics of DNA detection by using capped nanopores in the glucose release experiment. b. Schematics of cap detachment and glucose release from the GUV in the presence of target DNA. c. Mean concentrations of release glucose calibrated for the baseline release rate, i.e., calibrated concentration of 0 represents the same release rate as in the control experiment with GUVs in buffer. \( N = 3, 4, 7, 4, \) and 4 replicates for the experiments with capped nanopores added to samples with 1 \( \mu \text{M} \), 250 nM, 20 nM, and 0 DNA key and the experiment with open nanopores, respectively. d. Calibrated concentrations of released glucose measured at shorter time after mixing. Error bars in (c-d) are standard errors of the mean.

To test whether this proposed scheme, we measured the kinetic response of glucose concentrations when different concentrations of DNA key was added along with 2.5 nM cholesterol-modified capped nanopores to the GUV solution. We measured the time-lapsed bulk glucose concentrations within the first 8 hours of incubation and after sonication. To account for the variations in the total number of GUVs used in each experiment, we calculated the calibrated concentrations of released glucose by subtracting the baseline (in buffer) release rate from the measured concentration at each time point (Fig. 4.3c). The distinctively above-baseline glucose release responses at and after 1 hour represented positive detections of the DNA key species at as low as 20 nM concentration.

We then sought to find whether the assay could distinguish between the target DNA and other DNA species, and if so, whether the detection could be made faster than 1 hour. Thus, the glucose release experiment was repeated with either 20 nM DNA key or 1 \( \mu \text{M} \) nonmatching strand which would not interact with the capped nanopore. The glucose concentration measurements were made at 15 minutes and 30 minutes after mixing. The 20 nM DNA key triggered a glucose concentration response above the baseline release even at both time points.
while the nonmatching DNA did not (Fig. 4.3d), suggesting the assay could make a 15-minute positive detection. The conversion of 20 nM single-stranded DNA input to a response of 0.19 mM average glucose concentration increase in 15 minutes and 0.37 mM average glucose concentration increase in 30 minutes indicate 9,500-fold and 18,500-fold signal amplifications, respectively. The observation that a much higher concentration of nonmatching DNA strand did not trigger a response suggested the amplification and detection was specific to the target DNA sequence.

We have developed a DNA nanopore-based biosensor system, coupled with a commercial glucose meter, for detecting specific DNA sequences. The thousands-fold amplification of nanomolar analytes upon one-pot mixing and 15-minute isothermal incubation demonstrated ease of use, rapid response, and high sensitivity, making it a promising point-of-care diagnostic device. Our system can be potentially coupled with an isothermal nucleic acid amplification reaction to further improve the sensitivity. For example, adding a recombinase polymerase amplification (RPA) reaction95 that first amplify the lower concentration of nucleic acid to nanomolar level can lead to detection of attomolar nucleic acid species. Further, previous studies showed proteins154 and ATP155 can be transduced into DNA outputs with DNA aptamers. Thus, incorporating such DNA aptamer systems into our nanopore uncapping scheme would create biosensors for detections of a broad range of analytes.

Aiming at developing the DNA nanopore biosensor for detecting protein species, we made initial efforts on integrating a thrombin-binding DNA aptamer to our nanopore system. The new design was based on the protein-oligonucleotide signal exchange process previously reported95 (Fig. 1.6). By redesigning the toehold-mediated strand displacement reactions that were used to transduce the aptamer-thrombin bindings to a DNA output strand, we could then
create a system in which the output strand now had the same sequence as the uncapping strand, and the concentration of the uncapping strand would depend on the thrombin concentration. The redesigned reversible toehold-mediated strand displacement reactions would lead to a decreasing concentration of the DNA key when an increasing concentration of thrombin were being recognized. Thus, a higher concentration of thrombin would be reflected as a decreasing DNA key concentration in our system.

To characterize the response of the DNA key concentration to thrombin concentrations, we used a fluorescence assay (see Methods) to quantify the responses in DNA key strand (output strand) concentration when 0 – 200 nM thrombin were added to 50 nM DNA aptamers and transduction strands. As expected, DNA key concentration decreased with increasing thrombin concentration. The aptamer transduction reactions where different concentrations of thrombin were added all reached equilibrium very fast – within a few minutes when the fluorescence measurements were initiated. The causes for the increases in DNA key concentration after t = 1 h in the experiment where no thrombin was added were still not understood. Since 20 nM DNA key strand was able to induce above-baseline glucose release, and 80 nM thrombin was transduced to 20 nM DNA key strand output in the aptamer assay, the DNA nanopore biosensor should be able to detect the presence of over 80 nM thrombin when the aptamer transduction system was integrated. Further experiments are required to characterize the uncapping yields and glucose release with the thrombin aptamers.
Figure 4.4. Plot of DNA key concentration responses to different initial concentrations of thrombin for 50 nM DNA aptamer and transduction strands after 1-hour incubation at room temperature. The DNA key concentrations were calculated by calibrating the fluorescence intensity measurements.

4.4 Supplementary Information

4.4.1 Calibrate fluorescence intensities to DNA key concentrations

The fluorescence assay was used to determining time-lapsed response of DNA key concentration to different concentrations of thrombin (Fig. 4.4) by measuring the fluorescence due to the FAM fluorophore labeled on Z strand when separated from the quencher on the O strand. In order to relate the measured fluorescence intensities to O strand (DNA key) concentrations, we adapted a calibration process developed by Agrawal and Schulman\textsuperscript{95}. Briefly, we used a DNA strand (Z*) that is completely complementary to Z. When Z* was added to OZ complex, Z* would displace O from OZ complex, which separated the quencher and FAM fluorophore. Since this reaction is irreversible, the concentration of one of the products, ZZ*
complex, would equal to both the other product, DNA key, and the input concentration of Z*.
Thus, using different concentrations of Z* in this reaction and measuring the fluorescence responses could establish a relation between fluorescence intensities and DNA key. We added different final concentration of Z* (0, 25, 50, 75, 100, 150, 200, 300 nM) to a final concentration of 300 nM pre-annealed OZ. The fluorescence counts of the mixture was monitored in a plate reader (see Methods). The fluorescence counts had minimal changes within 30 minutes when incubated at 37°C during measurements, indicating the reaction equilibrium was reached. As expected, the fluorescence count was linearly correlated to input concentration of Z* (Fig. S4.1).

Assuming both unquenched and quenched FAM fluorophore contributed to the measured fluorescence counts, while the quenched fluorophore yielded a much lower fluorescence count, the measured fluorescence count reflected the sum of two linear functions, one of ZZ* (unquenched) concentration and the other one of OZ (quenched) concentration.

\[ f = a \times [ZZ^*] + b \times [OZ] + c \]  

(20)

where \( f \) is the measured fluorescence count (A.U.), \( a \) is the fluorescence parameter of ZZ*, \( b \) is the fluorescence parameter of OZ, and \( c \) is the background fluorescence. Because the sum of the concentrations of OZ and ZZ* is a constant, equal to the initial OZ concentration (300 nM). Thus, performing regression that fit Equation 20 to the fluorescence measurements resulted in

\[ a = 163.9 \frac{\text{A.U.}}{\text{nM}}, b = 7.7 \frac{\text{A.U.}}{\text{nM}}, \text{and } c = 2.0 \text{ A.U.} \]
Figure S4.1. Fluorescence calibration curve of FAM fluorophore.

The equation of the line is:

\[ y = 156.26x + 2306.1 \]

\[ R^2 = 0.9967 \]
Chapter 5: DNA Nanostructure that Self-heals in Serum

5.1 Introduction

DNA serves as a powerful and versatile programmable building block for bottom-up-based nanofabrication. One of the main challenges to the use of DNA nanostructures in drug delivery and for building biosensors is their rapid degradation in cell culture or in vivo by nucleases, which may be released as dying cells burst or be secreted. Fetal bovine serum (FBS), which contains various nuclease enzymes, at 37 °C is commonly used as a model system for understanding how DNA nanostructures might function in in vitro cell culture or in vivo and for characterizing their degradation. Typically, unmodified DNA nanostructures are completely degraded within 24 h when incubated in 10% FBS. A variety of approaches have been reported to enhance stability of DNA nanostructures in serum. Cassinelli et al. reported cyclization of DNA strands formed DNA nanostructures with enhanced exonuclease resistance. Coating DNA nanostructures with cationic polymers, lipid bilayers, or oligolysine-PEG polymers has also been reported to shield DNA nanostructures from enzymatic activity. Those reported strategies focused on protecting DNA nanostructures to improve their stability in biological environments. However, chemical modifications or coatings of either DNA strands or nanostructures must be devised specifically to achieve this protection, which for some modifications is labor- or cost-intensive. Modifications to DNA nanostructures, such as the conjugation of DNA to other charged molecules, could also compromise the biocompatibility of DNA materials which would be undesirable for some applications.

Here we develop a way to significantly extend the lifetime of a model DNA nanostructure through a self-repair process. When nuclease causes defects in the DNA
nanostructure during degradation process, the same monomers that the nanostructure consists of incorporate into the defect sites and replace damaged monomers in the nanostructure. Although DNA nanostructures protected by other approaches would inevitably go through the complete degradation process, the introduced self-repair process induces a repair rate that may fully counteract the degradation rate and thereby has the potential to create a dynamic system in which the DNA nanostructure concentration is maintained for long-term operation. Further, such a process does not depend on chemical modification or coating of the nanostructures and in many cases may work with unmodified DNA, making it potentially compatible with current approaches for large-scale DNA nanostructure synthesis.\textsuperscript{157,158}

We use this self-repair strategy to extend the lifetime of DNA nanotubes in serum. DNA nanotube structures have shown great promise as carriers for drug delivery, due to their high aspect ratio and encapsulation capacity\textsuperscript{159,160} and have also been used as components of tissue scaffolds.\textsuperscript{156} We demonstrate how DNA nanotubes can overcome damage in serum induced by nuclease degradation through self-repair, specifically, the incorporation of DNA nanotube monomers from solution. Monomers can incorporate within the nanotube body or at nanotube ends, allowing for repair of either lattice defects or decreases in overall nanotube length. This process is modeled after the dynamic assembly and repair of self-assembled structures and cellular architecture observed in living cells that can allow a cell to live for months or years, even though the lifetime of individual proteins ranges from hours to days.\textsuperscript{161,162} A simple model that we developed shows how the repair process we demonstrate can lead to increases in nanotube lifetime in serum. This model further suggests how, by properly tuning the dynamics of repair, it should be possible to achieve DNA nanostructure lifetimes of months or longer, much longer than those that might be achieved by relying on the chemical protection of DNA.
5.2 Methods

Materials

Tris/acetate/EDTA (TAE) buffer was purchased from ThermoFisher Scientific. Dulbecco’s Modified Eagle’s Medium (DMEM), magnesium acetate tetrahydrate, nickel(II) sulfate, tris-HCl, sodium chloride, and Tween-20 were purchased from Sigma-Aldrich. Fetal bovine serum (FBS) was purchased from Gibco. Polymer succinimidyl valeric acid PEG20k (PG1-SVA-20k) was purchased from NANOCS. Amicon ultrafiltration device was purchased from Fisher Scientific. Glass bottom dishes with 50 μm grids (81148) were purchased from ibidi.

Conjugation of PEG to DNA Tiles

The central strand of each DNA tile had a 5′ Cy3 or Atto488 fluorescent dye modification for fluorescence imaging of nanotubes and a 3′ primary amine modification for covalent conjugation with PEG20k-SVA (Supplementary 5.4.1). The DNA strand and PEG20k-SVA were conjugated by preparing a mixture of 50 μM of the DNA strand and 2 mM PEG20k-SVA in PBS buffer. The mixture was agitated overnight at room temperature. The DNA-PEG20k conjugate was then PAGE gel-purified. The concentration of purified conjugate was determined by comparing the fluorescence of conjugates against the fluorescence of a ladder of concentrations of fluorescent central strand of DNA tile using a MX3005P qPCR System (Agilent).

Assembly of Seeded Nanotubes to be Anchored to Glass

The 7,240bp M13mp18 scaffold strand was purchased from Bayou Biolabs. All other DNA was purchased from Integrated DNA Technologies, Inc. Nanotube seeds and inactive DNA tiles were prepared and annealed separately. The nanotube seed assembly mixture consisted of 5
nM scaffold strand, 200 nM staple strand mix, 100 nM adapter mix, 25 nM attachment strand mix, and 300 nM biotin linker strand mix (Supplementary 5.4.4). Inactive DNA tiles were prepared by mixing the six inactive tile strands (sequences, Supplementary 5.4.1.3) including PEG20k conjugated central tile strand, each at 350 nM concentration. All mixtures were prepared in TAE Mg²⁺ buffer (40 mM Tris-Acetate, 1 mM EDTA) with 12.5 mM magnesium acetate added.

Both seed and inactive tile assembly mixtures were subjected to a thermal annealing ramp with an Eppendorf Mastercycler according to the following program: incubate at 90 °C for 5 min, decrease to 45 °C at 1 °C/min, incubate at 45 °C for 1 h to allow formation of seed origami and inactive tiles, and decrease to 37 °C at 0.1 °C/min. After annealing, nanotube seeds were separated from excess staple strands using a 100k MWCO Amicon Ultra centrifugal filter device (Millipore). Following purification, 10 μl of 1μM Atto647-modified DNA strand was added to 40 μl purified nanotube seed sample and incubated at room temperature for 15 min to allow fluorescent labeling of seeds. The concentration of fluorescently labeled seeds was set to 1.6 nM, determined by measuring the concentration of a stock solution by counting the number of seeds per field of view (86 μm × 86 μm) after imaging them on a glass slide with fluorescence microscope (Olympus IX71) and then diluting this solution appropriately. A total of 1.8 μl of these fluorescently labeled seeds and 0.8 μl of tile activation strand (10 μM) were then added to 17.4 μl of the annealed tile sample. The sample was incubated at 37 °C for at least 15 h for nanotubes to grow.

Glass Surface Treatment and Attachment of Seeds to Glass
For all time-lapse experiments, seeded nanotubes were anchored via biotin-NeutrAvidin linker chemistry to passivated 0.17 mm-thick glass. The glass was passivated by attaching a biotin-labeled PEG-silane monolayer (Supplementary 5.4.4), after which NeutrAvidin protein (ThermoFisher Scientific 31000) was then added. A biotinylated DNA “universal biotin attachment strand” was then added to the passivated glass and incubated at room temperature for 15 min. The surface was then washed with TAE Mg$^{2+}$ buffer three times. Seeded nanotubes with six linker strands complementary to the universal biotin attachment strand were then added into the dish and incubated at room temperature for 10 min. The glass was then washed with TAE Mg$^{2+}$ buffer three times to wash away unanchored nanotubes before starting the experiment. In experiments in which DNA nanotubes were detached from the dish, an extended universal biotin attachment strand with a 5’ toehold domain not complementary to the linker strand was used to allow liftoff of seeded nanotubes by adding a displacement strand fully complementary to the extended universal biotin attachment strands (Supplementary S5.4.2).

**Fluorescence Microscopy**

For time-lapse experiments in which nanotubes were characterized at 12 h intervals, nanotubes anchored on glass were washed before each imaging step to reduce background fluorescence by pipetting 400 μl TAE Mg$^{2+}$ buffer onto the dish and removing it three times. In wide-field fluorescence microscope experiments, samples were imaged on an inverted microscope (Olympus IX71) using a 60×/1.45 NA oil immersion objective lens. At the initial time point, four locations indicated by the grid markings printed on the dish under bright field were identified to allow imaging of the same locations at subsequent time points. To estimate
nanotube lengths, one image of seeds and five images of nanotubes were captured at each location.

For confocal fluorescence microscope experiments, samples were imaged on an inverted microscope with a spinning disk unit (Zeiss Axio Observer Yokogawa CSU-X1M Spinning Disk Confocal) using a 63×/1.4 NA oil immersion objective lens. Seeds were imaged using a 633 nm diode laser, original nanotubes were imaged using a 488 nm diode laser and added monomers were imaged using a 561 nm diode laser. At each imaged location, one image of seeds was captured. Images of the two types of monomers were captured simultaneously in a burst of 20 time-series images using dual cameras.

Replenishment of Medium and Tiles during Time-Lapse Experiments

Tiles for experiments in which the serum-supplemented medium-contained DNA tiles were prepared by annealing 3.6 μM of each of the strands for inactive DNA tiles (Supplementary 5.4.3) using the annealing program in Seed annealing protocol (Supplementary 5.4.3.2). Every 12 h, following washing the dish with TAE Mg²⁺ buffer and fluorescence imaging, 50 μl of the annealed inactive DNA tiles was added to the dish sample. Activation strand (10 μM) and 4.2 μM adapter strands were added to the dish sample for desired final concentrations. Two-hundred fifty microliters of 12% FBS-supplemented DMEM medium was added for a total volume of approximately 300 μl. The dish sample was incubated at 37 °C in a temperature-controlled glove box (Coy labs) between imaging, washing, and buffer replacement steps.

Image Processing and Characterization of Nanotube Lengths
Composite two-color images of seeded nanotubes from time-lapse experiments were created by merging two grayscale filter images (Atto647 and Cy3). The same image of the seeds was matched with each of the images of nanotubes to create five images for each location at each time point. To maximize the accuracy of the length determination process, the contrast of each color (seed and nanotube labels) in composite image stacks was enhanced by linear histogram stretching using ImageJ software. Because we could only assess the 2D projection of nanotube length as we imaged the diffusing nanotubes through the bottom of glass dish using epifluorescence, the best estimation of nanotube lengths was made by measuring a nanotube’s length in each of the five images captured around a particular time point and selecting the longest of these lengths. The apparent length of a seeded nanotube in a given image was measured by manually drawing segmented lines along the nanotube curves, from the seed to the tip of nanotube, in ImageJ software. Seeded nanotubes whose measured lengths were less than 0.5 μm (about 3 pixels) were counted as having length 0.

Composite images from time-lapse tile incorporation experiments, performed on a spinning disk confocal microscope, were produced likewise by merging the one image of seed (Atto647) with each of the pairs of 20 dual-camera images of nanotubes (Atto488) and added tiles (Cy3) captured at each time point. The contrast of each color channel in composite images was enhanced by linear histogram stretching using ImageJ software. ImageJ’s “despeckle” algorithm, which replaced each pixel with the median value in its 3 × 3 neighborhood, was used to further reduce the background noise from free tiles in solution.

Simulation of Nanotube Growth and Repair
Stochastic kinetic simulations of nanotube degradation and repair were performed using the Gillespie algorithm for exact sampling of stochastic kinetic trajectories. Nanotubes were assumed to be six tiles in circumference and the length of the lattice in tiles was converted from the length in tiles assuming each tile row had a size of 14.3 nm. Rates of tile degradation and repair were as described in the text. The simulations were implemented in MATLAB as a reaction simulator on the cylindrical nanotube lattice that allowed reactions of tile degradation, tile repair at specified rates in units of per tile. Nanotube severing occurred automatically and irreversibly after all of the tiles in a given row were removed. Twenty nanotube degradation trajectories are shown in Figure 5.4a,d and Figure S5.12a,d and data from 2000 simulated nanotube degradation processes were simulated to produce the histograms and each curve in Figures 5.4b,c,e–g and S5.12b,c,e–g.

**Atomic Force Microscopy**

To characterize PEG-coated seeded nanotubes using atomic force microscopy, 5 μl of sample solution was added to a freshly cleaved mica surface on a puck with a Teflon sheet. A total of 20 μl of 5 mM nickel acetate-supplemented TAE buffer (TAE Ni²⁺ buffer) was then added and the sample was incubated for 5 min to allow the DNA nanostructures to adhere to the surface. The sample on mica was then washed twice with TAE Ni²⁺ buffer. Imaging was performed on a Dimension Icon (Bruker) using Scanasyst mode and sharp nitride lever tip (SNL, 10 C, Bruker) cantilevers. Images were flattened by subtracting a linear function from each scan line using the Nanoscope Analysis software. The lengths and widths of structures were measured using full width at half-maximum of AFM section profile.
5.3 Results and Discussion

DNA Nanotubes Self-Assemble from PEG-Conjugated DNA Tile Monomers

DNA tiles can assemble via Watson–Crick hybridization of their sticky ends to form nanotubes. Seeds are DNA origami structures that present a DNA template that DNA tiles or DNA nanotube ends can attach to via sticky end hybridization. DNA nanotubes were initially formed by isothermal incubation of DNA tile monomers formed from unmodified DNA (Fig. S5.1) with DNA origami seeds \(^\text{89,90}\) (Fig. 5.1b) at 37 °C. Nanotubes several microns in length with attached seeds formed during incubation, which we will refer to as seeded nanotubes.

**Figure 5.1.** DNA nanotubes self-assemble from DNA tiles via hybridization of complementary single-stranded DNA sticky ends. A DNA origami seed is a thermally stable structure that presents the facet of a growing nanotube using a set of adapter structures that co-assemble with the folded DNA origami body of the seed; tile adapters form a domain on one end of the seed that serves as a template for DNA nanotube growth. To prevent DNA nanotubes from sticking to the passivated glass surface in the presence of FBS,
one of the strands in the DNA tile is conjugated to a PEG polymer (gray chain). The same DNA strand also has a fluorescent dye to allow visualization of nanotubes via fluorescence microscopy. See Materials and Methods and Supplementary 5.4.1–5.4.3 for sequences and synthesis/assembly protocols. b. Schematic showing the fate of DNA nanotubes in serum-supplemented medium at 37 °C. Although DNA nanotubes degrade rapidly under these conditions, when DNA tiles that make up the DNA nanotubes are also present, these monomers can repair damaged nanotubes, extending nanotube lifetimes. c. Two-color fluorescence image of a seeded, PEG-modified nanotube. DNA nanotube monomers are labeled with Cy3 dye (green), seed with Atto647 dye (red). Scale bar, 1 μm. d. Atomic force micrograph of a seeded DNA nanotube with a PEG coating with average width of 24.7 ± 2.8 nm. Scale bar, 50 nm. e. Schematic showing how nanotubes are anchored by their seeds to a glass surface. To characterize change in nanotube lengths of populations of nanotubes and individual nanotubes over time, seeded nanotubes are anchored to a passivated glass surface through binding of a biotin labeled DNA strand on the seed to NeutrAvidin protein linked to biotin-PEG-silane monolayer on the glass surface (Supplementary 5.4.4). f. Multicolor fluorescence image of PEG-coated seeded nanotubes anchored to a passivated glass surface. Scale bar, 5 μm.

To study the stability of the nanotubes in serum, we incubated the nanotubes in 10% FBS and 12.5 mM magnesium chloride-supplemented Dulbecco’s Modified Eagle Medium (DMEM), which we will refer to as serum-supplemented medium, at 37 °C. The supplemented magnesium ions were necessary to maintain the shape of DNA origami structures. We characterized the stability of nanotubes by measuring changes in the two-dimensional (2D) projection lengths of nanotubes over time, after anchoring seeds of seeded nanotubes to the surface of a passivated glass.
Figure 5.2. Kinetic measurements of nanotube self-healing and degradation in serum. a. Average nanotube 2D projection lengths after different incubation times at 37 °C in serum-supplemented medium and in TAE Mg²⁺ buffer, normalized as fractions of average nanotube 2D projection lengths before incubation. Error bars are 95% confidence intervals. Helper strands (see text) comprise tile adapter strands (Figure 1) and tile activation strand (Supplementary 5.4.1). For t = 0, 12, 24, 36, 38, 60, 72, 84, and 96 h, N = 330, 269, 268, 270, 247, 219, 219, 215, and 215, respectively, for the matching tiles + helper strands group; N = 327, 288, 288, 284, 276, 260, 285, 257, and 267, respectively, for nonmatching tiles + helper strands group; N = 309, 185, 161, 132, 111, 92, 67, and 67, respectively, for helper strands group; N = 219, 173, and 152, respectively, for no DNA added group; N = 312, 214, 194, 164, 176, 154, 151, 138, and 128 for buffer group. b. Cumulative distributions of 2D projection lengths of DNA nanotube at 0, 24, and 48 h. Error bars are 95% confidence intervals. c. Multicolor fluorescence images of end-anchored seeded nanotubes with a PEG coating at different incubation time points with 267 nM matching tiles and helper strands added and with no DNA added. DNA nanotubes are labeled with Cy3 dye (green), seeds with Atto647 dye (red). Scale bars, 5 μm.
We found that aggregation and nonspecific interactions prevented characterization of the anchored nanotubes during incubation. Whereas the PEG monolayer that passivated the glass slide prevented DNA from sticking to the glass surface in standard buffers, in serum-supplemented medium we observed that nanotubes and monomers adhered to the surface over time (Fig. S5.2a). We also observed that DNA nanotubes quickly aggregated when incubated in test tubes with serum-supplemented medium, suggesting that proteins present in cell culture medium could cause undesired hierarchical interactions between nanotubes (Fig. S5.2b).

To prevent these interactions and to make it possible to characterize how the structure of anchored nanotubes changed over time in the presence of FBS, we developed a modified type of DNA nanotube by conjugating a 20 kDa molecular weight polyethylene glycol (PEG) polymer to one of the strands of the DNA nanotube monomers (Figure 5.1a, Fig. S5.3). The position of the conjugation was chosen so that the PEG chain protruded out of the nanotube. Atomic force and fluorescence micrographs confirmed that these modified monomers self-assembled to form nanotubes with attached seeds under the isothermal annealing conditions used to assemble unmodified nanotubes Figure 5.1c,d). We found that these PEG-coated nanotubes could be anchored to glass surfaces without interacting with the glass surface or aggregating (Figure 1e,f). The PEG-coated nanotubes achieved 2D projection lengths of several microns on average (Figure S5.8), making it possible to study the breakdown process using fluorescence microscopy.

**Breakdown of PEG-Coated DNA Nanotubes in Serum**

We first measured the degradation process of PEG-coated seeded nanotubes in serum-supplemented medium at 37 °C by following a population of end-anchored nanotubes over time.
The serum-supplemented medium was refreshed every 12 h after images were captured so that enzymes in the serum remained active throughout the experiment. The rate of degradation of the DNA nanotubes was characterized by measuring the 2D projection lengths of the nanotubes at the start of the experiment and at 12-h intervals, and then comparing average length at each time point to the initial average length. When DNA nanotubes were anchored and incubated in serum-supplemented medium at 37 °C, they completely disappeared (i.e., their average length was 0) after 24 h. This rate of degradation is consistent with the observed complete degradation of DNA octahedra,11 DNA triangular prisms83, and DNA tetrahedra163 in 10% FBS within 24 h. In contrast, PEG-coated seeded nanotubes anchored and incubated in TAE Mg²⁺ buffer at 37 °C, in which buffer was refreshed every 12 h, sustained 85% (±10%) of their initial average 2D projection lengths at 24 h and 63% (±10%) at 96 h. The slow reduction in average nanotube length over time could be a result of melting as monomers can detach from nanotubes at nanotube ends and are not replaced when no free monomers are in the solution.90 The significantly different rates at which the lengths of PEG-coated DNA nanotubes decreased suggested that although nanotubes could be slowly melted in serum due to the absence of free monomers, enzyme activity in serum played a predominant role in degrading the DNA nanotubes.

**DNA Tile Monomer Incorporation Slows down Nanotube Degradation**

DAE-E DNA tile nanotubes can assemble through a dynamic process of monomer attachment and detachment.88,90,165 We hypothesized that a similar dynamic assembly process could counteract degradation by allowing monomers to incorporate at the sites of enzyme-
induced defects as well as at nanotube ends. In such a case, freely diffusing DNA tiles in solution could repair the structures by replacing damaged tiles in the nanotubes.

To determine whether such a repair process might be feasible, we first asked whether free monomers might be able to incorporate within an existing nanotube as well as at nanotube ends. We grew DNA nanotubes from monomers labeled with one fluorescent dye and then incubated them with monomer tiles labeled with a second dye so that incorporation after growth in TAE Mg\(^{2+}\) buffer could be visualized (Supplementary 5.4.10). Monomers with the second dye were annealed in inactive form to prevent their assembly, and a DNA strand to activate tiles was added when the tiles with the second dye were added to the assembled nanotubes labeled with the first dye (Materials and Methods, Supplementary 5.4.1, 5.4.3, 5.4.11). We found that for some combinations of dyes, monomers were incorporated within nanotubes in addition to at nanotube ends (Fig. S5.12), suggesting that monomer incorporation could occur at sites within nanotubes.

We next studied the effects of additional free monomers on the lifetime of PEG-coated DNA nanotubes in serum-supplemented medium by adding annealed DNA tiles in an inactive form into the solution to a final concentration of 267 nM at the start of the experiment. A DNA strand, which we called activation strand, was then added to the mixture to transform the added tiles into an active form. To ensure that the tiles in solution could bind to the seeds and that nanotubes would not become irreversibly attached from seeds, we added adapter strands (which form the seed’s interface to the tiles) to the mixture along the activation strand (Supplementary 5.4.1). We referred to the activation strands and adapter strands that were added to the solution collectively as helper strands. The medium and new tiles were refreshed every 12 h. Under these
conditions, the lifetime of nanotubes was dramatically longer than the lifetime of nanotubes incubated in serum without additional DNA; nanotubes incubated with tiles and helper strands were 40% (±7%) as long after 96 h as they were at the start of experiment, whereas nanotubes incubated without tiles or helper strands had degraded completely by 24 h (Figure 5.2a). Until the incubation time of 60 h, the DNA nanotubes in “matching tiles + helper strands” group had statistically indistinguishable fractional average lengths as the DNA nanotubes in “buffer” group had, suggesting the repair of nanotubes by free tiles in serum could fully counteract the degradation of nanotubes by enzymes for over 2 days. In addition, although the degradation of nanotubes without DNA being added occurred precipitously, the decay of nanotubes in the presence of tiles and helper strands was gradual; the distribution of nanotube lengths measured changed only slowly over time.

The DNA added to the solution that the nanotubes were incubated in could have decreased the rate of nanotube degradation either by repairing the nanotubes or by serving as a competing substrate for nuclease degradation, thereby slowing down the rate of nanotube degradation. To verify that the replacement of damaged monomers by undamaged monomers was important for extending the lifetime of nanotubes in serum, we next tested how adding different types of DNA to the surrounding solution changed nanotube degradation rates. Adding only the helper strands resulted in longer nanotube lifetimes than adding no DNA at all, but the lifetimes of nanotubes were significantly shorter than the lifetimes when tiles were also included; the majority of nanotubes were less than 1 μm in length after 48 h (versus fewer than 25% at the start of the experiment) (Figure 5.2b) and more than 90% of the nanotubes’ total length was lost after 84 h of incubation (Figure 5.2a). However, in this case the total concentration of DNA in solution was less than the total amount of DNA present in the solution of helper strands and tiles.
during the experiments testing repair. To test the extent to which this lower total DNA concentration in solution was a factor in the decreased nanotube lifetime, we next characterized nanotube lifetime in a solution containing the same total concentration of DNA as the nanotube tiles and helper strands solution, but we replaced the DAE-E nanotube tiles with DAE-E tiles with sticky-end sequences that were not complementary to the sticky ends on the nanotubes. The noncomplementary sticky-end sequences prevented these tiles from incorporating into the nanotubes. We called these tiles nonmatching tiles. Nanotubes incubated in the solution of helper strands and nonmatching tiles also had shorter lifetimes and a greater reduction in average nanotube length than nanotubes incubated in a solution of helper strands and tiles that could incorporate into nanotubes. In addition, when both helper strands and nonmatching tiles are present, nanotubes had longer lifetime compared to nanotubes incubated in only helper strands, suggesting that increasing the amount of added DNA also slows degradation by serving as competing substrates to nuclease.

**Incorporation of DNA Tiles into Nanotubes during Degradation**

The observation that the presence of matching tiles increased nanotube lifetime more than the presence of nonmatching tiles at the same concentration suggested that the incorporation of tiles into nanotubes could increase their lifetime in serum. We thus next sought to determine whether matching tiles incorporated into nanotubes during the degradation process. To make it possible to visualize the incorporation of tiles during degradation, we prepared nanotubes assembled from tiles labeled with one dye (Atto488) and incubated these nanotubes in serum-supplemented medium containing matching tiles labeled with a different dye (Cy3). This pair of dyes was chosen to allow simultaneous imaging of the original nanotubes and added tiles using a
dual camera on a spinning disk confocal microscope that could simultaneously capture images of the two types of tiles within individual nanotubes during the growth process. Comparisons of images of the same anchored nanotubes after different incubation times showed how the matching tiles could extend nanotubes and that matching tiles could incorporate into the bodies of existing nanotubes (Fig. 5.3a). In some cases, the nanotubes anchored to the surface eventually consisted entirely of the matching tiles that were in the incubation solution (Figure 5.3a, nanotube 2).
Figure 5.3. Incorporation of DNA Tiles into Nanotubes. **a.** Multicolor confocal micrographs and corresponding schematics of two example-anchored DNA nanotubes (blue, Atto488) before and at different time points during incubation with free nanotube monomers (green, Cy3) in serum-supplemented medium. The free monomers can both incorporate within nanotubes and extend existing nanotubes. Seeds are not shown. Scale bars, 2 μm. **b.** Four sets of multicolor fluorescence micrographs showing nanotubes that were incubated in serum-supplemented medium for 24 h while anchored by their seeds to a glass-bottomed dish. After incubation, the anchor was detached from the surface using a strand displacement process (Supplementary S5.4.2) and the solution containing the detached nanotubes was deposited onto glass slides for fluorescence imaging, as illustrated in the schematic. Images of repaired nanotube are overlays of the images from the channels used to measure the original nanotubes (monomers were labeled with Atto488), free monomers present in the incubation solution (Cy3), and the seed (Atto647). Scale bars, 2 μm.

Whereas simultaneous two-color imaging made it possible to follow the degradation and recovery of a single nanotube over time, it was in general difficult to determine how nanotube tiles incorporated within a nanotube using this technique because many parts of the nanotube, which could move in three-dimensions, were not in sharp focus. To better visualize how matching tiles became incorporated into nanotubes during degradation, we anchored nanotubes to a glass bottom dish with a DNA linker that could be detached using a DNA strand displacement process (Supplementary 5.4.2). We incubated nanotubes with this linker on dishes in serum-supplemented medium at 37 °C for 24 h in the presence of Cy3-labeled matching tiles; the solution was replaced with fresh medium and tiles at $t = 12$ h following our previous experiments. The nanotubes were then detached from a dish by adding the displacement strand, and the resulting solution was plated on microscope slides. Images of these nanotubes showed
that tiles incorporated not only at the ends of nanotubes but also within the original nanotubes (Fig. 5.3b). The incorporation of tiles in small regions of the original nanotubes suggesting that the matching DNA tiles in solution could repair damaged DNA nanotubes.

A Model of the Dynamics of DNA Nanotube Degradation and Repair

Our observations of matching tile incorporation into nanotubes during degradation demonstrated that tiles incorporated into and extended nanotubes when longer nanotube lifetimes were observed. To understand how and the extent to which the incorporation of DNA tiles or other DNA species might affect the kinetics of nanotube degradation in the presence of nucleases, we created a simple model of nanotube degradation and repair. To focus on the role of repair rather than nanotube regrowth, the model included only incorporation of tiles into an existing nanotube and ignored the possibility of nanotube growth.

The model represents the nanotube as a cylindrical lattice of monomers connected according to the connectivity imposed by nanotube sticky ends. Nanotubes were assumed to be six monomers in circumference, in accordance with previous measurements of the circumference of seeded DNA tile nanotubes\(^{88,89}\). Within the model, a nanotube initially starts out at a particular length; the number of rows was chosen assuming that if monomers are 14.3 nm long,\(^{88}\) each row of monomers contributes the same to total nanotube length. Nucleases were assumed to degrade nanotubes by removing individual tile monomers at a given stochastic (microscopic) reaction rate. Although the removal of a small number of monomers would create holes in that lattice but leave it connected, the removal of too many would cause the nanotube to sever. In our simulation, we assumed that severing would occur when the lattice of tiles that formed the
nanotube became disconnected, forming two nanotubes that start and end respectively at the point at which the discontinuity arose.

Figure 5.4. Simulated nanotube degradation and repair and differences in the dynamics of nanotube degradation and nanotube coupled degradation and repair observed in experiments. **a.** Example lengths of nanotubes over time predicted by a stochastic kinetic model of nanotube degradation in which individual monomers are removed from the nanotube at a rate of 0.03/monomer/h. **b.** Distribution of times at which individual simulated nanotubes became short enough to be considered degraded. **c** Fractions of nanotubes remaining in simulation over time for different monomer degradation rates. **d−f.** Simulations and results as in (a−c) except that monomers can be degraded and gap sites in the lattice neighboring at least two intact monomers can be repaired by the incorporation of a tile from solution. The repair rate is 0.12/gap/h in (d,e) and as shown in (f). **g.** Predicted fractions of nanotubes remaining over time made using simulations from (a−f) to compare with the results of experiments made by choosing appropriate degradation and repair rates; for experiments with baseline degradation or no DNA added
(0.03/monomer/h), reduced rates of degradation that would be expected for when helper strands
(0.009/monomer/h) and helper strands and monomers with unrelated sequences (0.007/monomer/h) were
added to the buffer and when nanotubes were both being degraded (0.007/monomer/h) and repaired
(0.009/gap/h) when helper strands and matching tiles were present in buffer. h. Time-lapse multicolor
fluorescence micrographs showing seeded nanotubes incubated in serum-supplemented medium at 37 °C.
When no DNA was added, the DNA nanotube remained intact for a few hours before rapidly
disassembling. When 267 nM matching tiles were present in solution, refreshed every 12 h, a typical
DNA nanotube was not completely degraded over several days but decreased gradually in length. Scale
bars, 1 μm.

To directly compare the model’s results to our experiments, we simulated the reactions of
tile degradation by cleavage in a stochastic kinetic simulation (see 5.4.9) and tracked how the
lengths of anchored nanotubes changed over time. (The dynamics of the nanotube fragments that
became disconnected from the anchored structures were not simulated after their disconnection.)
During the simulated degradation of a population of nanotubes that were initially 5 μm long,
nanotubes remained at their initial length for some period of time but then suddenly severed.
After an initial severing event, a nanotube rapidly severed again and again until it was short
enough to be considered fully degraded (Fig. 5.4a). This type of relatively sudden decrease in
nanotube length was observed in experiments where DNA was not added to the serum during
incubation (Fig. 5.4h, S5.16).

The distribution of nanotube severing times in serum without additional DNA present
predicted in the model was qualitatively different than the distribution of times of degradation of
individual tiles. Tile degradation events should be broadly (i.e., exponentially) distributed,
following the memoryless nature of the degradation reaction. In contrast, severing events were
tightly clustered in a peaked distribution, presumably because severing happens when the fraction of defects in the tube approaches the critical fraction where the nanotube’s lattice would no longer be connected.

To compare the predictions of the model with our experiments, we assumed that a nanotube would be considered degraded in micrographs when it became too short to be seen clearly, about 30 rows of monomers in length. The distribution of times at which a 5 μm long tube reached this state in simulations of nanotube degradation are shown in Fig. 5.4b. These decay times are clustered in a peaked distribution. Changing the rate of decay, as might occur with the introduction of a competitor species for nucleases, such as the helper strands or tiles with random sequences, would be expected to slow degradation. Simulations suggest that slowing the rate of degradation would shift the average time at which degradation occurred but would still cause most nanotubes to persist and then degrade within a relatively short period (Fig. 5.4c).

Qualitatively different behavior was observed in simulations where both degradation of monomers and lattice repair, modeled as reincorporation of an intact tile at a site where a tile had left, was allowed. Instead of nanotube severing events being clustered in a narrow range of time, nanotubes could sever and then persist for a long time before severing again (Fig. 5.4d). The resulting times at which nanotubes reached the fully degraded states were thus spread across a distribution with a long tail (Fig. 5.4e), in contrast to the distribution of times that was clustered around a particular peak time observed when nanotube monomers could be degraded and repair could not occur (Fig. 5.4b). Repair thus allows some nanotubes to persist for very long times even when on average nanotubes still decay quickly. Our simulations also predict that increasing the repair rate could allow most nanotubes to last for very long times.
While decreasing the monomer defect rate effectively delays the time to full degradation by a linear factor, increasing the repair rate slows the rate of degradation much more rapidly and extends the range of lifetimes by introducing a long tail to the lifetime distribution (Fig. 5.4f). These qualitative changes in the distribution of nanotube lifetimes persist in a simulation of nanotubes with the same distribution of initial lengths as those used in this the time-lapse degradation experiments (Fig. S5.11).

A degradation process in its simplest form might be viewed by analogy to a Poisson process, in that decreasing the effective rate of repair increases the expected time before a certain number of defects arise by a linear factor. In contrast, a degradation process in which repair was faster on average than degradation might be viewed as a reverse-biased random walk, such that the expected time to sever at a particular point is on order exponential in the number of monomers that must be degraded in a local region of the nanotube lattice to cause severing. Increasing the ratio of the monomer repair rate to monomer degradation rate has the effect of increasing the base of this exponent. Because the number of monomers that must be degraded in the nanotubes, we have studied is large (on order 6), the inclusion of repair dramatically affects how the lifetime of a structure can be manipulated. For example, simulations predict that increasing the repair rate by just a factor of 2 for a process that allows the majority of nanotubes to survive for 2–3 days could allow the majority of nanotubes to survive for more than a month (Fig. 5.4f). Thus, the use of repair could be an important strategy for structures and devices that are designed to persist for months or years in an environment where degradation of components is likely to occur.

In this paper, we report a dynamic DNA nanotube system that allows the degradation of DNA nanostructures in serum to be reversed through in situ self-repair. While PEG-coated DNA
nanotubes, self-assembled from monomers, were completely degraded after 24 h incubation in serum-supplemented medium at 37 °C, their lifetimes were significantly increased when free monomers were introduced in the solution. Free monomers repaired the degrading nanotubes by replacing damaged monomers that made up the nanotubes and joining onto nanotube ends. The increase in nanotube lifetime was enhanced by the fact that monomers can serve as a competitor to DNA nanotube as enzyme substrates. We developed a stochastic kinetic model to simulate the dynamics of nanotube degradation and repair. The predictions of this model matched our experimental results and showed how inclusion of repair rate in a degrading system could allow nanostructures to persist for a long time.

Another important challenge to using DNA nanostructures for in vivo applications is that the high concentration of magnesium ions needed to stabilize DNA may be incompatible to in vivo systems. Approaches developed by other investigations might help stabilize DNA origami in physiological conditions. For instance, Shih et al. reported oligolysine-PEG conjugate coating prevents DNA origami structures from low-salt denaturation. Cassinelli et al. reported using click chemistry to form DNA catenanes allow nanostructures to remain stable without salts. Tuukkanen et al. reported DNA nanostructures deposited through a spray-coating method are stable in salt-free solution. Those reported approaches may be used in tandem with our approach of increasing nanostructure lifetime in serum to advance the potential of DNA nanostructures for use in vivo applications or other environments where degradation of DNA occurs.

5.4 Supplementary Information

5.4.1 Nanotube tile design and conjugation of PEG to DNA
M13mp18 scaffold strand was purchased from Bayou Biolab. All other DNA strands used in this study were synthesized by Integrated DNA Technologies, Inc. (IDT). The DNA nanotube tile and adapter strands were desalted while Cy3, Atto647 and Atto488 fluorophore-labeled strands, biotin-labeled, and amino-modified strands were HPLC purified. Concentrations for DNA strands were determined either by measuring absorbance at 260 nm wavelength or by using IDT’s stated yields to determine solution concentrations.

**Conjugation of PEG chains to DNA tiles**

The tile design and DNA sequences are the same as described in 2.4.1. After adding serum-supplemented medium to the seeded nanotubes anchored to glass cover slips, we observed that DNA nanotubes formed from the tiles in Fig. S2.1 adhered to the glass and to one another, making it difficult to observe degradation and also suggesting that the function of these structures would be limited by unintended interactions (Fig. S5.1). To reduce these effects, we developed a modified tile that presented a polyethylene glycol (PEG) chain. To make these tiles, we modified the central DNA tile strand (SEs_3-5’Cy3 in Figure S1) to present a 5-base thymine spacer with a 3’-end primary amine at its end (Fig. S5.1). The amine-modified DNA strand, ‘SEs_3-5’Cy3_3’amine’, was purchased from IDT in PAGE purified form. To attach a PEG polymer to this strand, we reacted the amine on 3’ end of the central DNA tile strand with N-hydroxysuccinimide (NHS) functionalized polyethylene glycol (PEG) valeric acid with molecular weight of 20,000 Da (NANOCS, PG1-SVA-20K) as described in the main text Methods.
Figure S5.1. Multicolor fluorescence images showing seeded nanotubes without PEG coating. 

a) anchored to a glass dish after incubation in 10% FBS supplemented DMEM at 37°C for 12 hours. The anchored nanotubes adhered to glass surface and to each other. DNA structures and tiles that appeared to be degraded also adhered to surface, creating background noise in the image. DNA nanotubes are labeled with Cy3 dye (green) and seeds with Atto647 dye (red). 

b) incubated in 10% FBS supplemented DMEM at 37°C for 90 minutes in a 200 μl PCR tube then 6 μl of nanotube solution was plated onto a glass slide for imaging. Undesired clusters of nanotubes are observed, suggesting that unmodified nanotubes incubated in test tubes also aggregated. DNA nanotubes are labeled with Cy3 dye (green) and seeds with ATTO647 dye (red). Scale bars, 5 μm.
Primary amine-modified SEs tiles are conjugated to succinimidyl valeric acid PEG with a molecular weight of 20 kDa (PEG20k-SVA).

SEs_3-5’Cy3_3’amine:
/Cy3/CCAGAACGGCTGTGGCTAAACAGTAACCGAAGCACCAACGCTTTTTT/AmMO/
/AmMO/ denotes an amino group covalently attached to the 3’ end of the DNA.

**Design of inactivated tiles and a corresponding activation strand**

To ensure that the tiles that were added to the serum supplemented DMEM had not themselves assembled into nanotubes before the start of the experiment, we modified the tiles to create tiles that were annealed in an inactive form, adapted from design of Zhang et al. These inactive tiles could then be activated, *i.e.*, reach a conformation that allowed assembly into nanotubes, by a strand-displacement reaction with an activation strand (Figure S5.3). The inactive tiles are designed such that one of the sticky ends is double-stranded, preventing the tiles from forming a lattice by sticky end joining. The activation strand, ‘SEs_activation’, upon
addition to the solution, displaces the ‘SEs_inactive_strand5_right’ strand and exposes a single-stranded sticky end where a double-strand end was previously. The resulting products have four exposed sticky ends, allowing assembly of DNA nanotubes.

Figure S5.3. Schematic of the reaction in which the activation strand reacts with an inactive tile by displacing the strand that covers one of the sticky ends. The resulting reaction “activates” the tile.
Sequences of SEs_1, SEs_2, and SEs_4 tile strands are listed in S1.1. Sequence of central tile strand is given in S2.4.2.

SEs_inactive_strand5_left: CGATGACCTGCTTC
SEs_inactive_strand5_right: GTTACTGTTTAGCCTGCTCTACCAGAC
SEs_activation: GGTTACTGTTTAGCCTGCTCTA

5.4.2 Seed design and sequences

The sequences of staple strands used to fold DNA nanotube seeds in this work are the same as those in Mohammed et al\textsuperscript{90}. The following seed adapter strands, added along with staple strands at annealing, were modified to present the sticky end sequences of the tiles used this study.
Figure S5.4. Schematic of the assembled adapter tiles for the seeds. The gray lines and their corresponding sequences are components of the M13mp18 scaffold.

Adapter strands sequences

AD1Se6bp_1-: AGGGATAGCAAGCCCAACACGTTGAGGACACTTGGAGGCTGCACTC
AD1_2Se6bp_3-: TGTCTTCACGTTGCTGGATGCCGATCCTACGACACCTCCAAG
AD1_2Se6bp_5-: CGCTGACTTGTCGTAGGATCGGCATCCAGATAGGAACCCATGTAC
AD2Se6bp_2-: CCAGAC GAGTGCAGAGTCAGCGTACCTC
Nanotube seeds were labeled with Atto647 fluorophore dyes for fluorescence imaging. The labeling system consists of 100 attachment strands, each of which contains a subsequence that binds to the section of the M13mp18 scaffold that is not folded by staples. The reminder of
the attachment strand binds to a labeling strand that has Atto647 fluorophore dye on the 5’ end, ‘labeling_strand_ATTO647N’. The sequences of the attachment strands are the same as those listed in Mohammed et al.\textsuperscript{103}

\begin{align*}
\text{labeling\_strand\_ATTO647N:} & \quad /5\text{ATTO647NN}/\text{AAGCGTAGTCGGATCTC}
\end{align*}

### 5.4.3 Preparing seeded nanotubes

**Preparation of nanotube seed annealing solution**

To assemble seeds, 50 μl annealing mixture was prepared containing M13mp18 scaffold, staple strands, adapter strands, fluorescent attachment strands and biotin attachment strands in 1x TAE Mg\textsuperscript{2+} buffer in the quantities shown below.

Recipe for preparing nanotube seed annealing solution

<table>
<thead>
<tr>
<th></th>
<th>Desired final concentration (nM or fold)</th>
<th>Stock concentration (nM or fold)</th>
<th>Volume added (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H\textsubscript{2}O</td>
<td></td>
<td></td>
<td>27.6</td>
</tr>
<tr>
<td>TAE Mg\textsuperscript{2+} buffer</td>
<td>1</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Seed staple mix (concentrations are per strands)</td>
<td>200</td>
<td>1389</td>
<td>7.2</td>
</tr>
<tr>
<td>Seed adapter strand mix (concentrations are per strand)</td>
<td>100</td>
<td>1000</td>
<td>5</td>
</tr>
<tr>
<td>M13 scaffold</td>
<td>5</td>
<td>100</td>
<td>2.5</td>
</tr>
<tr>
<td>Fluorescent attachment strand mix (concentrations are per strand)</td>
<td>25</td>
<td>1000</td>
<td>1.25</td>
</tr>
</tbody>
</table>
All of the following DNA strand mixes are prepared by dissolving DNA strands in MilliQ water:

Seed staple mix: Mixture containing all 72 seed strands at equal concentrations. Mixing individual seed staple strands (each stock at 100 µM) in equal volume makes the final concentration of staples in the mixture to be 1.389 µM (100 µM/72).

Seed adapter strand mix: solution containing all 24 seed adapter strands (as shown in Figure S5.4) in equal concentrations of 1 µM per strand.

Fluorescent attachment strand mix: solution containing 100 attachment strands in equal concentrations of 1 µM per strand for the fluorescence labeling of seeds. Sequences of fluorescent attachment strands are as given in Mohammed et al.¹⁰³

Biotin attachment strands mix: mixture containing all 6 biotin attachment linker strands (Sequences as described in Section S5.4).

**Seed annealing protocol**

The annealing protocol is the same as described in 2.4.3.

**Seed purification and fluorescent labeling**

Seeds were separated from excess staple, adapter, biotin attachment, and fluorescent attachment strands by the following purification process.
After annealing, 50 µl of seed solution and 350 µl TAE Mg2+ buffer were added to a 100kDa Amicon µltra-0.5mL centrifugal filter (UFC510096) and centrifuged at 3000 RCF for 4 min in a fixed-angle centrifuge. The sample was washed two more times by adding 350 µl TAE Mg2+ buffer into the remaining solution and repeating centrifugation. Purified seed solution was collected by spinning the inverted filter in a new tube. To fluorescently label the seeds, 12 µl of 1 µM Atto647 labeling strand was added to approximately 40 µl purified seeds collected from the filter unit. The mixture was incubated at room temperature for at least 15 minutes. The concentration of purified seeds was measured by adopting the method developed by Agrawal et al. After purification, an imaging solution was prepared by mixing 0.3 µL seed solution with 19.7 µl tile mix solution (containing 0.05 mg/ml BSA). 6 µl mixture was then transferred to a glass slide to be imaged under a fluorescence microscope with 60x objective. We continued to dilute the purified seeds until 100-200 seeds per field of view (87 µm x 87 µm) were observed, indicating an approximate seed concentration of 6pM in the imaged solution.

**Annealing inactive tiles**

DNA tiles were annealed separately from seeds before they were mixed with purified seeds. 88 µl of solution containing 398 nM of each of the inactive SEs tile strands (as listed in 5.4.1) in TAE Mg2+ buffer was annealed using the same annealing protocol as “seed annealing protocol”.

**Self-assembly of seeded nanotubes**

6 µl purified seeds, diluted to 0.8 nM concentration, prepared as in S3.1-3 were mixed with 88 µl of the annealed inactive tile solution prepared in “Preparation of nanotube seed
annealing solution” and incubated at 37°C. 4 µl of activation strand to a final concentration of 400 nM was then added to activate annealed tiles so that the tile concentration was 350 nM after the addition of purified seeds and the activation strands. The mixture was incubated at 37°C for at least 24 hours to allow the seeded nanotube to grow to their maximum lengths.

5.4.4 Anchoring seeds to and detaching seeds from passivated glass

![Diagram](image)

**Figure S5.5.** Schematic illustrations of the passivated glass surface and the anchoring of DNA nanotube seeds to the surface via biotin-NeutrAvidin chemistry. NeutrAvidin molecules were deposited on the treated glass surface. 6 biotin attachment linker strands (green strands) each were bound to the M13mp18 scaffold and to a universal biotin attachment strand (gray strand with biotin, pink). The universal biotin strands bound to NeutrAvidin molecules present on the glass surface.
Biotin attachment linker strands sequences for the nanotube seed:

Biotin_rightside_01: CTATTATTTCTGAAACATTTTCACATCGTCACTCCT
Biotin_rightside_02: CAGGAGGGTTGAGGCAGTTTTCACATCGTCACTCCT
Biotin_rightside_03: ATCAAGTTTGCCTTTATTTTCACATCGTCACTCCT
Biotin_rightside_04: GGTTTACCAGCGCCAATTTTCACATCGTCACTCCT
Biotin_rightside_05: TTTTAAGAAAGTAATTTTCACATCGTCACTCCT
Biotin_rightside_06: AAACGATTTTTTGTGTTTTTTCACATCGTCACTCCT

Universal biotin attachment strand: /5BiosG/AGGAGTGACGATGTG

/5BiosG/ denotes a biotin protein covalently conjugated to 5’ end of a DNA strand.

Protocol for NeutrAvidin presentation on the glass surface

Glass-bottom dishes with a 50 µm labeled grid (ibidi µ-Dish 35 mm, high Grid-50 Glass Bottom) were cleaned by sonication in 10% NaOH for 20 minutes. Dishes were then washed with water to remove residual NaOH followed by a methanol wash. 500 µl of 10 mg/ml biotin PEG silane MW 3400 (Biotin-PEGSIL-3400-500mg, Layson Bio) solution (in solvent containing 95% methanol, 4% acetic acid, and 1% water) was prepared and added to dishes. Dishes were then stored overnight in a fume hood with a Parafilm seal to prevent evaporation. The next day, dishes were first rinsed with methanol, then with water to remove residual methanol. The glass bottom surfaces of the dishes were blow dried with pressurized nitrogen. Dishes were baked in an oven at 90°C for 1 hour to cure and crosslink the biotin PEG silane. 500 µl of 1% BSA blocking solution (in TNT buffer) was added to dishes and incubated for 1.5 hours. Dishes were then washed with TNT buffer to remove excess blocker. 500 µl of 0.4 mg/ml NeutrAvidin (31000, Thermo Fisher Scientific) in TNT buffer was added to dishes and incubated for 30 minutes.
Dishes were washed with TNT buffer followed by 3 washes with TAE Mg\textsuperscript{2+} buffer. 500 µl of TAE Mg\textsuperscript{2+} buffer was added to dishes after the washing steps.

TNT buffer: 10 mM Tris-HCl, 0.1 M NaCl, 0.05% Tween-20, pH 7.5

**Design of detachable nanotube anchor**

The extended biotin attachment strand was designed by adding a 5-bp toehold domain to the universal biotin strand. In experiments where seeds were detached from the surface, seeds were anchored by hybridizing the extended biotin attachment strand on the surface rather than the universal biotin attachment strand. A biotin displacement strand, the full complement of the biotin attachment strand (excluding a spacer) was used to detach the reversibly anchored seeded nanotubes on passivated glass by binding to the extended biotin attachment strand and displacing the biotin attachment linker strands on seeds.

---

**Figure S5.6.** Schematic illustration showing a biotin attachment linker strand on a seed. The seed can be detached from an extended biotin attachment strand anchored on a passivated glass via a strand.
displacement reaction involving a biotin displacement strand. The biotin displacement strand (orange strand) hybridizes to a toehold on the extended biotin attachment strand (gray strand with a biotin, pink), and displaces the biotin attachment linker strand (green strand) on the seed. As a result, the seed detaches from the glass surface.

Extended biotin attachment strand: /5BiosG/TTTT GTGAG AGG AGT GAC GAT GTG
Biotin displacement strand: CACATCGTCACCTCCTCAC

5.4.5 Fluorescence images and length distribution of PEG-coated DNA nanotubes

Figure S5.7. PEG-coated seeded nanotubes. a. Wide-field fluorescence image of PEG-coated seeded nanotubes grown as described in SI 3 and then plated on a glass slide. DNA nanotubes are labeled with Cy3 dye (green) and seeds with Atto647 dye (red). Scale bar, 5 µm. b. Lengths of nanotubes in two fields on view were measured and the results are shown in a histogram graph of length distribution of seeded nanotubes with a PEG coating. N = 142.
5.4.6 Additional AFM images of PEG coated seeded nanotubes

![Sample AFM images of PEG coated DNA nanotubes. Scale bars, 200 nm.](image)

**Figure S5.8.** Sample AFM images of PEG coated DNA nanotubes. Scale bars, 200 nm.

5.4.7 Fluorescence images of nanotubes after different periods of serum-supplemented medium incubation
Figure S5.9. Additional fluorescence microscopy images showing the breakdown of end-anchored PEG-coated seeded nanotubes after different incubation times in four different conditions. DNA nanotubes are labeled with Cy3 dye (green), seeds with Atto647 dye (red). Scale bars, 5 μm.
5.4.8. Additional fluorescence confocal images of repaired nanotubes

**Figure S5.10.** Additional multicolor time-lapse fluorescence microscopy images of tile incorporation.

Free tiles (green, Cy3) joined and incorporated into original anchored nanotubes (blue, Atto488) incubated in serum-supplemented medium at 37°C. Scale bars, 2 μm.

5.4.9. Additional simulations of nanotube degradation and repair
Figure S5.11. Simulated degradation and repair of nanotubes. The initial distribution of lengths of nanotubes was chosen from the set of all nonzero initial lengths of nanotubes measured in the degradation experiments performed in this work. a-c. Repeat of the simulations in Fig. 5.4a-c using the length initial distribution of nanotubes from experiments. d-e. Repeat of the same simulations as shown in Fig. 5.4d-f using the initial distribution of nanotubes drawn from experimental measurements. The results show the same qualitative effects of degradation (a tendency for nanotubes to degrade within a clustered range of times) and repair (the creation of a long tail in the distribution of nanotube lifetimes that can dramatically extend the times over which nanotubes persist).

5.4.10. Incorporation of tiles into nanotubes in TAE Mg\(^{2+}\) buffer

In order to show that tiles in solution can incorporate within DNA nanotubes, we first studied how DNA tiles incorporate into DNA nanotubes in a standard buffer where degradation is not expected to occur. To distinguish tiles that were incorporated after the nanotubes were annealed from the tiles that became incorporated after assembly, the tiles present in the original anneal and the tiles added subsequently were labeled with different fluorescent dyes. To test
whether tiles would still incorporate despite the differences in tile interactions that might be caused by differences in fluorescent labeling, three different fluorescence schemes for labeling the nanotubes and free tiles in solution were tested. We compared growth and incorporation of Cy3-labeled nanotubes and Atto647-labeled free tiles, Atto647-labeled nanotubes and Cy3-labeled free tiles, and Cy3-labeled nanotubes and Atto488 labeled free tiles. In each fluorescently labeled tile, the fluorophore was present at the same location as in Cy3 labeled tiles (Fig. S5.2).

This comparison was done using seeded nanotubes without PEG coating. Seeds were annealed and purified following the steps described in S3.1-3. 18 µl inactive tiles (Fig. S5.2), were annealed at 167 nM concentration in TAE Mg²⁺ buffer using the annealing protocol in S3.2. Then, 1.6 µl purified seeds and 0.4 µl of activation strand to a final concentration of 200 nM were mixed with 18 µl of the annealed inactive tile solution. The tile concentration becomes 150 nM after addition of purified seeds and activation strand. The mixture was incubated at 37°C for at least 24 hours to allow all seeded nanotube to grow to their maximum lengths.

After preparing seeded nanotubes, we annealed 19.4 µl free tiles in their inactive form (Fig. S4) at 204 nM concentration following the same annealing protocol as described in S3.2. Then, 0.6 µl activation strand to a final concentration of 300 nM was added to the annealed tiles, which were kept in 37°C incubation. Right after adding the activation strand, 5 µl prepared seeded nanotubes were mixed with 15 µl activated free tiles so that free tile concentration became 150 nM after addition. The mixed solution was kept incubated at 37°C for at least 12 hours before imaging.

Incorporation of tiles into nanotubes was observed by plating 0.6 µl of each sample onto a bare glass slide and imaging using epi-fluorescence microscope (Olympus IX71). As shown in Figure S5.12, for each color scheme experiment, the free tiles both joined at the growing ends of
the existing nanotubes and incorporated within the existing nanotubes. The tiles added to the already assembled nanotubes also themselves assembled into new, unseeded nanotubes. The amount of tile incorporations differs among different color schemes, possibly because different fluorescent labeling molecules affected the kinetics of nanotube assembly, joining or monomer incorporation. We observed more tile incorporation into Cy3-labeled nanotubes by Cy5-labeled free tiles and into Cy5-labeled nanotubes by Cy3-labeled free tiles than into Cy3-labeled nanotubes by Atto488-labeled free tiles. Although the results suggested Cy3-labeled tiles and Cy5-labeled tiles were more compatible, we used Atto488 labeling and Cy3 labeling for the nanotubes and free tiles in the experiment that observed tile incorporation during degradation (Fig. 5.3), because this color combination allowed us to use a spinning disk confocal microscope that could capture the Atto488 and Cy3 channels simultaneously and because PEG-coated Cy3-labeled free tiles exhibited the least non-specific interaction with the surface of the three types of free tiles when incubated in serum-supplemented medium.
Figure S5.12. Fluorescence images of nanotubes grown in solution to which free tiles with different fluorescence labels are added. (a) Atto647-labeled (red) tiles incorporated into Cy3-labeled (green) nanotubes nucleated from Atto488-labeled (blue) seeds, (b) Cy3-labeled tiles incorporated into Atto647-labeled nanotubes nucleated from Atto488-labeled seeds, and (c) Atto488 labeled tiles incorporated into Cy3-labeled nanotubes nucleated from Atto647-labeled seeds. Scale bars 5 µm.

5.11 Inactive tiles do not form nanotubes
To verify that inactive tiles do not form nanotubes, 20 µl of Cy3-labeled inactive tiles at 300 nM concentration were annealed following the protocol described in 5.4.3, and incubated at 37°C for 30 hours before imaging with fluorescence microscope. To then verify that these tiles would form nanotubes after activation, 1.2 µl of activation strand at 10 µM concentration was added to 18.8 µl tile solution to reach a final concentration of 600 nM activation strand in solution. The mixture was incubated at 37°C for nanotube growth and imaged with fluorescence microscope after 22 hours.

Figure S5.13. Monochrome fluorescence images of tile activation. a) inactive tiles alone after incubation at 37°C for 30 hours and b) the same tiles formed nanotubes after addition of activation strand and subsequent 22-hour incubation at 37°C. No nanotubes formed from inactive tiles, but many nanotubes formed after the tiles were activated.

5.4.12 Nonmatching tiles

U tiles, which have the same structure and size but different sequences than SEs tiles (30), were added to nanotubes in serum-supplemented medium as part of control experiments to
test the effect of a given concentration of DNA on the rate of nanotube degradation. To ensure a consistent comparison between the effects of U and SEs tiles in solution on degradation rates, U tiles were, like SEs tiles, conjugated with PEG polymer molecules. Because U tiles have completely different sticky end sequences, they are not expected to interact with SEs tiles or DNA nanotubes consisting of SEs tiles. U tiles also do not have sticky end sequences that would allow them to interact with each other or form nanotubes alone after annealing.

**Figure S5.14.** Schematic showing the architecture of U tiles.

**U tile sequences:**

U_1: GTTAAGGACGACGCAATTCTCACATCGGACGAGTAG
U_2: ATCAGCCTACTCGTGGATCTATGCTTGC
U_3-5’Cy3-3’amine:

/56FAM/AGAATTGCGTGGATCTAGGCTTGCTCAGCTACCGGATGTTTTT/AmMO/

U_4: GTCTCTGGTTTCACCTTAACGCAAGC
U_5: ATAGATCCCTAGCAGACCTAGCAACCTGAAACC

/56-FAM/ denotes FAM fluorophore covalently attached to the 5’ end of DNA.

**5.4.13 Helper strand analogs without sequence interactions with seeds or nanotube tiles**
To ensure that the same amount of DNA was added to the serum-supplemented medium in the control group (‘nonmatching tiles and helper strands’) as in the experimental group (‘matching tiles and helper strands’) when the rate of nanotube degradation was measured, we designed DNA that matched the structure and total length of the helper strands in the experimental group but lacked sequences that allowed for binding interactions between the helper strands and seeded nanotubes. The helper strands used in the experiments include the activation and adapter strands. The activation strand added in the control group was the same strand used to activate inactive SEs tiles (S5.4.1). No interaction between this strand and the nanotubes was expected because the SEs tiles in the nanotubes anchored to the surface are already in the active form. The adapter strands used in place of the helper strands consisted of two domains. The first domain was complementary to adapter binding domain on seeds, and the second domain had randomly generated sequences so that added tiles would not interact with seeds. The adapter strands used in the other control group, where only helper strands were added to DNA nanotubes in serum-supplemented medium, were the same as the strands used in the experimental group and are listed in Section 5.4.2.

*Sequences of adapters with random sequence tile binding domain:*

AD1UEd_1_rand: GACACCGAAGCGGATGTGGAAGCACTAGCTCGCGAAAGCACGTAG
AD1_2UEd_3_rand: TTCCACATCCGCTCTGGCAGTCACCTCGCATCAGGCTAGTGC
AD2UEd_5_rand: ATTGAATTTCAGCCCGCTGATGCGAGGTGACTGCCAGCAGATGGT
AD1_2UEd_2_rand: TCTCTGACCATCTGTCCGTGTGCAGAAGC
AD5UEd_1_rand: GATGAGCATCGACGTGGCTTCACCTACTGCGTGAGATGCACGC
AD5_6UEd_3_rand: GAAGCCACGTCGAGACCAGTCCTACCACACAGCTCGCAGTAGGT
AD6UEd_5_rand: TAAAGTTTCCCAAAGACGAGCTGTTGGTGGTAGGACTTGCTCGCTATGCC
AD5_6UEd_2_rand: TCTCTGGGCACTAGCTGCTCATCGCAAGC
AD9UEd_1_rand: GTGCTCGACTGACACCGAATCCGTGGATGGCGTCGTGATGCAGGC
AD9_10UEd_3_rand: GATTCGGTGTCAGAGCTGGTTGGACTCATGCCGTCATCCACG
AD10UEd_5_rand: GCTCAACACCCAATGTACGGCATGAGTCCAACCAGCTACGACCTA
AD9_10UEd_2_rand: TCTCTGTAGGTCGTTCGAGCACGCAAGC

5.4.14 Nonmatching tiles do not incorporate into nanotubes

To verify that U tiles, used in the control group for the amount of DNA in the experiment, do not incorporate into SEs tile nanotube, we captured images of the samples with fluorescence microscope at each time point to characterize how FAM-labeled U tiles became incorporated (or not) into anchored SEs nanotubes in serum-supplemented medium. At each time point (t = 12h, 24h, 36h, …), after washing the dish with TAE Mg\textsuperscript{2+} buffer, one image in FAM channel was captured along with seeds and DNA nanotubes. As in other time-lapse experiments, fresh U tiles, helper strands, and serum supplemented DMEM were replenished to the dish after each imaging. At no time point were U tiles observed in nanotubes.
**Figure S5.15.** Fluorescence images of U tiles and multicolor images of seeded nanotubes on dish glass surface. Captured after buffer washing at different time points. U tiles are labeled with FAM (blue), nanotubes with Cy3 (green), and seeds with Atto647 (red). The images showed that all the U tiles added into the solution were washed away during buffer washing step, indicating that no U tiles incorporated into nanotubes after 12-hour incubations in serum at 37°C.

5.4.15 Additional fluorescence images of anchored DNA nanotube degradation in serum
Figure S5.16. Time-lapse fluorescence images showing degradation of seeded DNA nanotubes. Nanotubes are anchored on a glass coverslip incubated in serum-supplemented medium at 37°C. Nanotubes are labeled with Cy3 (green) and seeds with Atto647 (red). DNA nanotube disassembled rapidly from $t = 8$ hours to $t = 16$ hours.

5.4.16 Gel-electrophoresis of DNA nanotube with a PEG-20kDa coating

Succinimidyl valeric acid PEG-20kDa (Catalog # PG1-SVA-20k, Nanocs, New York, NY, USA) was conjugated to the central strand of the 6bp SEs tile using the same protocol as described in 5.4.2. The conjugated product was confirmed through electrophoresis using 7% PAGE gel (150V, 1 hour).

Figure S5.17. PAGE gel-electrophoresis image of the DNA-PEG=20kDa conjugation. The top row indicates conjugation products of PEG-20kDa and “SEs_3-5’Cy3_3’amine” DNA strand. The gel was
imaged under UV light after staining with SybrGold. The band near the top of the gel indicates the desired conjugated product, which was then extracted from the gel into MilliQ water.

5.4.17 DNA nanotubes with a PEG-40kDa coating

Before we adopted the strategy that coats the DNA nanotubes with PEG-20kDa, we tested coating the nanotubes with PEG-40kDa (polyethylene glycol of average molecular weight of 40 kDa). Fluorescence image showed DNA seeded nanotubes with a PEG-40kDa coating were formed (Fig. S5.18). However, the time-lapsed fluorescence microscopy experiment suggested all the nanotubes attached to a glass coverslip incubated in 10% serum was completely degraded within 6 hours (Fig. S5.19). Thus, the PEG-40kDa coating did not provide enhanced protection against enzymatic degradation compared to a PEG-20kDa coating.
Figure S5.18. Multicolor fluorescence image of nanotubes (Cy3, yellow) assembled on seeds (red, Atto647) with a PEG-40kDa coating. The nanotubes were formed following the protocol described in 5.4.3. Scale bar, 5 μm.

Figure S5.19. Fluorescence images of nanotubes with a PEG-40kDa attached onto a glass-bottom dish. Captured at a) t = 0 and b) t = 6 h. The nanotubes were incubated at 37°C in DMEM medium supplemented with 10% FBS. Scale bar, 5 μm.
Chapter 6: Future Directions

In this thesis, I designed and synthesized DNA-based nanopore and nanochannel that showed transport of small molecules. The transport of glucose from the vesicles through DNA nanopores, initiated by cap releasing upon DNA key capture, has allowed the development of a nanopore-based biosensor for nucleic acids. I showed the currently detection limit of the target nucleic acid was 20 nM. The sensitivity of detection is mostly limited by the baseline glucose release, caused by the leaks across vesicle membranes. Further reducing the leaks would enable measurements of smaller amount of nanopore-mediated glucose release, leading to detection of lower concentrations of target nucleic acids. One potential approach for developing a leak-free system is to use polymersomes\textsuperscript{167} or polypeptide vesicles\textsuperscript{168} to encapsulate glucose molecules, which showed much lower leakage of encapsulated molecules compared to lipid vesicles. These synthetic vesicles are also more chemically and mechanically stable than lipid vesicles, making them ideal materials for building biosensors with long storage lifetime. Nevertheless, whether and how DNA nanopores can incorporate into polymersomes or polypeptide vesicles have not been studied. Thus, further research is required to address this challenge.

I showed DNA nanochannels were able to self-heal in biological environment where enzymes in the serum actively digested the DNA structure, making nanochannels promising a material for building conduits that can enable molecular transport over longer distances with an extended structure lifetime. However, it remains unknown whether other types of molecules, such as nucleic acids, peptides, and proteins can be transported within the nanochannels. The inner diameter of 7 nm of the nanochannels should theoretically allow the transport of larger molecules, including folded proteins, but the transport needs to be confirmed and characterized.
through further experiments. The fluorescence-based dye influx assay used for studying TAMRA transport should be applicable to investigating the transport of other molecules if the molecules of interest can be fluorescently labeled. Alternatively, translocations through the nanochannels can be investigated on single-molecule level using an electrophysiological setup that monitors the conductance of nanopore-inserted membranes. If the capacity for transporting proteins or nucleic acids within the DNA nanochannels are established, the nanochannels can be potentially developed into microreactors for catalytic reactions or highly sensitive biosensors. The length of the nanochannel would lead to long residence time of the target molecules inside the channel while the interior decorations of functional groups can interact with the target molecules.

Another potential application is to use the DNA nanochannels for studying cell receptor activations. The nanochannels would serve as an interface between a living cell of interest and an abiotic device. After the nanochannels connect individual cell receptors of specific types on cell membranes and a device that can electronically control the flow of ligand, the temporally controlled ligand flow to the receptors can be used to study receptor activation kinetics when cellular responses are monitored. To build such a device, the one end of the nanochannel need to be positioned on and linked to a solid state nanopore, and the flow across the nanopore is to be controlled by voltage gating\textsuperscript{169,170}. This kind of combination of DNA nanopore and solid state nanopore has been reported, so similar techniques can be applied for the DNA nanochannel. Secondly, the other end of the nanochannel need to be specifically attached at or near the cell receptor of interest so that the flow of the ligand would create a high local concentration of ligand in close proximity to the receptor. Jia et al. has developed a method for attaching DNA nanotubes to cell membrane through antibody bindings\textsuperscript{101}. A similar approach may be used to attach the nanochannels to the target cell receptors. We envision the construction of such
nanofluidic electronic cell coupled device that can activate individual cell receptors will enable systematical probing of cell signaling and adaptive behaviors.
References


Yi Li

206-724-7778 | yli244@jhu.edu | www.linkedin.com/in/yli244/

Education
Johns Hopkins University
Baltimore, MD
Ph.D. in Chemical and Biomolecular Engineering, GPA: 3.97 / 4.0
Expected completion on May 15th, 2021
Advisor: Rebecca R. Schulman

University of Washington
Seattle, WA
B.S. in Chemical Engineering, GPA: 3.95 / 4.0
2012 – 2016

Research Interests
Developing assays and biosensors for detecting various biomolecules and DNA sequencing by exploiting biological membranes and DNA nanotechnology.

Research Experience
Johns Hopkins University
Baltimore, MD
Graduate Researcher Sep 2016 - present
• Characterized kinetics of nanostructure degradation and repair in the cell culture environment
• Developed assays to measure molecular transport through self-assembled nanofluidic channels
• Engineering an isothermal amplification method for in situ detection of nucleic acids

University of Washington
Seattle, WA
Undergraduate Researcher Sep 2014 – Jun 2016
• Investigated metabolic pathways of microbial strains using isotope labeling
• Extracted and purified metabolites from bacteria cultured in various conditions
• Quantified metabolites with LC-MS and validated metabolic kinetics by modeling

Work Experience
Amgen Thousand Oaks, CA
Process Development Graduate Intern Jun 2020 – Sept 2020
• Explored clone selection optimization opportunities in the Cell Line Development team
• Developed an automated image-based workflow for analyzing CHO cell phenotypes
• Built machine learning algorithms that improved the predictability of fed-batch clone performance based on cell growth and morphology data

Teaching and Mentoring Experience

Johns Hopkins University

Teaching Assistant, Dynamic Modeling and Control
Sep 2019 – Dec 2019
• Led weekly recitation sessions and prepared assignments for 60 undergraduate students to learn designing control systems for chemical processes through modeling
• Gave a guest lecture on process dynamics and control in biomolecular systems

Teaching Assistant, Advanced Chemical Reaction Engineering in Practice
Jan 2018 – May 2018
• Prepared practice sessions with real-world problems related to reaction system design for over 70 graduate students
• Created and graded course assignments and tests to ensure student understood materials

Graduate Mentor, Chemical and Biomolecular Engineering
Jan 2018 – Dec 2019
• Mentored a graduate student in M.S.E program in a research project on patterning nanostructures on the gold surface with photolithography technique
• Mentored two undergraduate students in implementing experiments and image data acquisition using fluorescence microscopy
• Guided the students in the preparation of presentations of research findings

Skills and Coursework

Laboratory:

Protein expression & purification | Cell culture | Fluorescence microscopy
Surface chemistry | AFM | Electron microscopy
Molecule labeling | Chromatography | Photolithography

Coursework:

Metabolic System | Interfacial Science | Transport Phenomena
Biomolecular System Design | Python Carpentry | Advanced Thermodynamics

Computer: Python, Java, Matlab, Comsol, Adobe Illustrator

Publications

2017 Fu, Y., Li, Y., & Lidstrom, M. The oxidative TCA cycle operates during methanotrophic growth of the Type I methanotroph Methylomicrobium buryatense 5GB1. *Metabolic Engineering*, 42, 43-51. DOI: 10.1016/j.ymben.2017.05.003

**Leadership Experience**

**Johns Hopkins University**

*Representative* of Graduate Student Liaison Committee  
March 2019 – Present
- Organized social and professional development events to support engineering graduate student communities
- Planned and participated in new student orientations

**University of Washington**

*Vice President* of American Institute of Chemical Engineers (AIChE)  
Sep 2015 – Jun 2016
- Outreached to local companies for recruitment sessions at the university
- Organized social events for the regional AIChE conference

*Resident Advisor*  
Sep 2014 – Jun 2015
- Facilitated the holistic development of residents through programming
- Referred, advised, and supported residents with academic and social resources

**Honors and Awards**

2016 Bernice Frank Scholarship
2016 University of Washington Chemical Engineering Honors Program
2015 Mary Gates Research Scholarship
2015 Henry K. Benson Scholarship

**Presentations**

**Oral presentation**  
Aug 2019 Yi Li, Rebecca Schulman. DNA Nanostructure that Self-heal in Serum. The 25th International Conference on DNA Computing and Molecular Programming; Seattle, WA.

**Poster presentations**  


Dec 2019 Yi Li, Rebecca Schulman. Molecular transport through self-assembled DNA nanofluidic channels. MADNANO Conference by National Institution of Standards and Technology; Gaithersburg, MD.
Sep 2019  Yi Li, Rebecca Schulman. Molecular transport through self-assembled DNA nanofluidic channels. Asilomar Bioelectronics Symposium; Pacific Grove, CA.

Aug 2019  Yi Li, Rebecca Schulman. DNA Nanostructures that Self-Heal in Serum. 25th International Conference on DNA Computing and Molecular Programming (DNA25); Seattle, WA.

May 2019  Yi Li, Rebecca Schulman. Molecular transport through self-assembled DNA nanofluidic channels. Institute for Nanotechnology Nano-Bio Symposium; Baltimore, MD.

Apr 2019  Yi Li, Rebecca Schulman. Molecular transport through self-assembled DNA nanofluidic channels. 63rd Annual Meeting of the Biophysics Society; Baltimore, MD.

Jul 2018  Sisi Jia, Yi Li, Naresh Dhanasekar, Rebecca Schulman, Self-targeting biotic-abiotic interface. DARPA Young Faculty Awards; Arlington, VA.

Apr 2018  Yi Li, Rebecca Schulman. Evaluate and improve DNA nanotube serum stability. 16th Annual Conference Foundations of Nanoscience (FNANO); Salt Lake City, UT.


May 2015  Yi Li, Yanfen Fu, Mary E. Lidstrom. Exploring the Metabolism of Methylomicrobium buryatense 5G. University of Washington Undergraduate Symposium; Seattle, WA.