REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
05-04-2023	Final Report	1-Aug-2014 - 31-Jul-2022

4. TITLE AND SUBTITLE
Final Report: Programing Biomolecular Nanodevices For Targeted Immune Cell Recognition and Payload Delivery

5a. CONTRACT NUMBER
W911NF-14-1-0507

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102
611103

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of California - San Francisco
Office of Sponsored Research
3333 California Street Suite 315
San Francisco, CA 94143 -0962

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
66074-BB-PCS.6

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Shawn Douglas
UU	UU	UU	UU		19b. TELEPHONE NUMBER 415-502-1947

RPPR Final Report
as of 05-Apr-2023

Agency Code: 21XD

Proposal Number: 66074BBPCS **Agreement Number: W911NF-14-1-0507**
INVESTIGATOR(S):

Name: PhD Shawn Douglas
Email: Shawn.Douglas@ucsf.edu
Phone Number: 4155021947
Principal: Y

Organization: **University of California - San Francisco**
Address: Office of Sponsored Research, San Francisco, CA 941430962
Country: USA
DUNS Number: 094878337 EIN: 946036493
Report Date: 31-Oct-2022 Date Received: 05-Apr-2023
Final Report for Period Beginning 01-Aug-2014 and Ending 31-Jul-2022
Title: Programing Biomolecular Nanodevices For Targeted Immune Cell Recognition and Payload Delivery
Begin Performance Period: 01-Aug-2014 **End Performance Period:** 31-Jul-2022
Report Term: 0-Other
Submitted By: PhD Shawn Douglas Email: Shawn.Douglas@ucsf.edu
Phone: (415) 502-1947
Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 3 **STEM Participants:** 6

Major Goals: The major goal of this project is to lay the groundwork for developing next-generation therapeutic devices using biologically inspired nanotechnology. Our vision is to create, de novo, nanoscale devices from DNA, RNA, and proteins that rival the power and sophistication of natural machinery found in living organisms. Such devices, if realized, would enable a variety of life-saving and life-improving technologies that seem feasible based on first principles and physical laws, but are unprecedented in humans. Examples include safely activating and re-targeting immune cells to attack and clear specific cell types (e.g. cancer cells) that otherwise evade the immune system, or safely suppressing the immune system to avoid host rejection of a transplanted organ. Longer-term applications of our nanostructures could include nanoscale scaffolding and stem-cell stimulation for limb regeneration.

To achieve our vision, we must reverse-engineer methods for bottom-up self-assembly of nanodevices that are capable of performing specific functions in living organisms. We are primarily focused on using DNA as a molecular building block for constructing these nanoscale devices, and leveraging proteins to perform biochemistry. We have previously developed initial prototypes of devices capable of targeted delivery of protein payloads to specific cell types. We aimed to extend and improve these prototypes. Once we build and test suitable designs in vitro, we aim to scale up production for in vivo studies. We planned to characterize their performance in living organisms, testing their toxicity and efficacy and optimizing them through design iterations.

Accomplishments: See Uploaded PDF.

Training Opportunities: 1. Dr. Douglas has mentored three graduate students and two postdocs individually, discussing career development, research topics, work plans, and supervising fellowship proposals, manuscript drafts, and presentations.

2. The team conducted weekly group meetings for research discussions and presentation rehearsals. Graduate students participated in Software Carpentry workshops, while postdocs trained in grant and fellowship writing, including a successful Department of Energy grant application.

3. Graduate students Parsa Nafisi and Suraj Makhija attended the 2017 FNANO conference, presenting their work in a poster session.

4. Postdoctoral researcher Dr. Tural Aksel trained in electron microscopy and established TALE expression, purification, and related techniques in the lab, and trained Suraj Makhija in these skills. All project participants trained in DNA origami design using the Cadnano software.

5. Graduate students Parsa Nafisi, Suraj Makhija, and Dr. Tural Aksel attended the 2018 FNANO conference, presenting their work in a poster session. Dr. Aksel further trained in electron microscopy, and all project participants continued training in DNA origami design using Cadnano.

6. Dr. Konlin Shen, Dr. Rui Dong, and Dr. Xiao Huang gained training in various DNA origami techniques, including design, analysis, folding preparation, application, and attachment strategies. All project participants trained in DNA origami design using the Cadnano software.

Results Dissemination: # Public Talks and Seminars

Dr. Douglas presented the results of this project at the following meetings:

2019 GenScript Double Helix Symposium, San Francisco CA
2019 Designing Materials That Compute, Army Research Office, Durham NC
2018 Engineering Biomolecules Mini Symposium, University of Oregon OR
2018 Bioengineering Speaker Series, Caltech, Pasadena CA
2017 DoD Science Technology Innovation Exchange (STIx) Washington DC
2015 Communications Design Group (CDG) All-Hands Meeting, Los Angeles CA
2015 Techonomy Bio Converence, Mountain View, CA
2015 Bloomberg BusinessWeek Design Conference, San Francisco CA
2014 University of California San Diego, Bioengineering Seminar, San Diego CA
2018 ACM Symposium on Computational Fabrication Pittsburgh PA
2018 14th Annual Conference Foundations of Nanoscience Snowbird UT
2018 InterPlanetary Festival, Santa Fe Institute, Santa Fe NM
2016 World Congress of Science & Factual Producers, Stockholm, Sweden
2016 ACSB Annual Meeting, Nanotechnology Subgroup, San Francisco CA
2016 Ten Years of DNA Origami, Caltech, Pasadena CA
2015 Brazilian Materials Research Society Meeting, Rio de Janeiro, Brazil
2014 Annual Meeting of the Biophysical Society of Japan, Sapporo, Japan
2014 International Conference on DNA Computing and Molecular Programming (DNA20), Kyoto, Japan
2014 Cluster of Excellence Center for Advancing Electronics Dresden, Seminar Series, Dresden, Germany
2014 Max Planck Institute of Molecular Cell Biology of Genetics, Seminar Series, Dresden, Germany

Software workshops

We co-organized a training workshop in which 32 participants from all over the world met at UIUC and received hands-on training in DNA origami design and simulation using our tools. The tutorial materials from that workshop have been made available online: https://www.ks.uiuc.edu/Training/Workshop/Urbana2018c/

Collaborations

We have applied the software tools and algorithms developed as part of this project to provide highly optimized DNA origami designs for several collaborators, including:

Björn Högberg, Dept. of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm Sweden
Chenxiang Lin, Cell Biology and of Biomedical Engineering, Yale University, New Haven CT
Paul Rothemund, Caltech, Pasadena CA
Ron Vale, UCSF, San Francisco CA
Yifan Cheng, UCSF, San Francisco CA
Orion Weiner, UCSF, San Francisco CA
Dmitry Lyumkis, Salk Institute, La Jolla, CA

Honors and Awards: 2018 UCSF Excellence in Teaching Award
Haile T. Debas Academy of Medical Educators

2017 Presidential Early Career Award for Scientists and Engineers (PECASE)
Office of the President (Barack Obama)

2015 CAREER Award
National Science Foundation

2014 Pew-Stewart Scholar in the Biomedical Sciences
Pew Charitable Trusts

Protocol Activity Status:

RPPR Final Report
as of 05-Apr-2023

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Shawn Douglas
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Tural Aksel
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Erik Navarro
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Pablo Damasceno
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Other Professional
Participant: Nick Fong
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Undergraduate Student
Participant: Passa Pungchai
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: High School Student
Participant: Tara Saxena
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
National Academy Member: N

RPPR Final Report
as of 05-Apr-2023

ARTICLES:

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Nucleic Acids Research
Publication Identifier Type: DOI Publication Identifier: 10.1093/nar/gkw208
Volume: 44 Issue: 11 First Page #:
Date Submitted: 4/27/17 12:00AM Date Published: 6/1/16 2:00PM
Publication Location:
Article Title: Folding complex DNA nanostructures from limited sets of reusable sequences
Authors: Stefan Niekamp, Katy Blumer, Parsa M. Nafisi, Kathy Tsui, John Garbutt, Shawn M. Douglas
Keywords: self-assembly, DNA, nanotechnology
Abstract: Scalable production of DNA nanostructures remains a substantial obstacle to realizing new applications of DNA nanotechnology. Typical DNA nanostructures comprise hundreds of DNA oligonucleotide strands, where each unique strand requires a separate synthesis step. New design methods that reduce the strand count for a given shape while maintaining overall size and complexity would be highly beneficial for efficiently producing DNA nanostructures. Here, we report a method for folding a custom template strand by binding individual staple sequences to multiple locations on the template. We built several nanostructures for well-controlled testing of various design rules, and demonstrate folding of a 6-kb template by as few as 10 unique strand sequences binding to 10 ± 2 locations on the template strand.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Synthetic Biology
Publication Identifier Type: DOI Publication Identifier: 10.1093/synbio/ysy015
Volume: 3 Issue: 1 First Page #:
Date Submitted: 9/4/18 12:00AM Date Published: 8/3/18 9:00PM
Publication Location: Oxford, United Kingdom
Article Title: Construction of a novel phagemid to produce custom DNA origami scaffolds
Authors: Parsa M Nafisi, Tural Aksel, Shawn M Douglas
Keywords: DNA origami, phagemid, DNA nanotechnology
Abstract: DNA origami, a method for constructing nanoscale objects, relies on a long single strand of DNA to act as the ‘scaffold’ to template assembly of numerous short DNA oligonucleotide ‘staples’. The ability to generate custom scaffold sequences can greatly benefit DNA origami design processes. Custom scaffold sequences can provide better control of the overall size of the final object and better control of low-level structural details, such as locations of specific base pairs within an object. Here, we report the construction of a novel phagemid, pScaf, to produce scaffolds that have a custom sequence with a much smaller fixed region of 393 bases. We used pScaf to generate new scaffolds ranging in size from 1512 to 10 080 bases and demon- strated their use in various DNA origami shapes and assemblies. We anticipate our pScaf phagemid will enhance develop- ment of the DNA origami method and its future applications.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
Acknowledged Federal Support: **Y**

RPPR Final Report
as of 05-Apr-2023

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: eLife
Publication Identifier Type: DOI Publication Identifier: 10.7554/eLife.68311
Volume: 10 Issue: 10 First Page #: e68311
Date Submitted: 12/10/21 12:00AM Date Published: 6/1/21 9:00PM
Publication Location:
Article Title: Tight nanoscale clustering of Fc-gamma receptors using DNA origami promotes phagocytosis
Authors: Nadja Kern, Rui Dong, Shawn M Douglas, Ronald D Vale, Meghan A Morrissey
Keywords: Macrophage, cell signaling, DNA origami
Abstract: Macrophages destroy pathogens and diseased cells through Fc? receptor (Fc?R)-driven phagocytosis of antibody-opsonized targets. Phagocytosis requires activation of multiple Fc?Rs, but the mechanism controlling the threshold for response is unclear. We developed a DNA origami-based engulfment system that allows precise nanoscale control of the number and spacing of ligands. When the number of ligands remains constant, reducing ligand spacing from 17.5 nm to 7 nm potently enhances engulfment, primarily by increasing efficiency of the engulfment-initiation process. Tighter ligand clustering increases receptor phosphorylation, as well as proximal downstream signals. Increasing the number of signaling domains recruited to a single ligand-receptor complex was not sufficient to recapitulate this effect, indicating that clustering of multiple receptors is required. Our results suggest that macrophages use information about local ligand densities to make critical engulfment decisions, which has
Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Proc Natl Acad Sci U S A.
Publication Identifier Type: DOI Publication Identifier: 10.1073/pnas.2109057118
Volume: 118 Issue: 40 First Page #:
Date Submitted: 12/10/21 12:00AM Date Published:
Publication Location:
Article Title: DNA origami patterning of synthetic T cell receptors reveals spatial control of the sensitivity and kinetics of signal activation
Authors: Rui Dong, Tural Aksel, Waipan Chan, Ronald N. Germain, Ronald D. Vale, Shawn M. Douglas
Keywords: T cell receptor, cell signaling, DNA origami
Abstract: Receptor clustering plays a key role in triggering cellular activation, but the relationship between the spatial configuration of clusters and elicitation of downstream intracellular signals remains poorly understood. We developed a DNA-origami-based system that is easily adaptable to other cellular systems and enables rich interrogation of responses to a variety of spatially defined inputs. Using a chimeric antigen receptor (CAR) T cell model system with relevance to cancer therapy, we studied signaling dynamics at single cell resolution. We found that the spatial arrangement of receptors determines the ligand density threshold for triggering and encodes the temporal kinetics of signaling activities. We also showed that signaling sensitivity of a small cluster of high-affinity ligands is enhanced when surrounded by non-stimulating low-affinity ligands. Our results suggest that cells measure spatial arrangements of ligands and translates that information into distinct signaling dynamic
Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
Acknowledged Federal Support: **Y**

WEBSITES:

URL: https://www.nanotk.app/
Date Received: 08-Feb-2023
Title: Nano Toolkit
Description: This web application provides a cloud-based interface to the tools and algorithms developed during this project.

RPPR Final Report
as of 05-Apr-2023

Partners

,

I certify that the information in the report is complete and accurate:
Signature: Shawn Douglas
Signature Date: 4/5/23 9:12PM

Accomplishments

1. We developed DNA origami architectures to reduce the component count by up to 93% for enhanced manufacturing scalability.

We published a paper, **Folding complex DNA nanostructures from limited sets of reusable sequences**, by S Niekamp et al, Nucleic Acids Res. 44:e102, describing a successful new approach to create DNA nanostructures of similar size and complexity to published methods, but with 7- to 15-fold reduction in the number of components necessary. That is, we have created DNA origami nanostructures that fold from 1 scaffold and 10 to 20 unique staples. Our approach requires the synthesis of a custom scaffold for each device, but allows for individual staples to bind in multiple locations on the same scaffold (Figure 1).

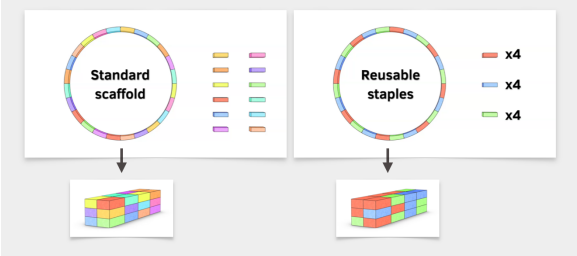


Figure 1. Schematic representations of the standard DNA origami approach (left) and our new reusable-sequences method (right)**.** Our new approach reuses the same sequences throughout a custom scaffold, allowing only four copies of 3 staple sequences to be used to fold a similar block shape that originally required 12 staple sequences.

We designed a set of 24-helix bundles with 6-kilobase scaffolds, and tested versions designed to fold using 10, 15 or 20 unique staple sequences (Figure 2). For comparison, a similar shape designed using the DNA origami method requires ap- proximately 150 unique staples. Thus, for the designs with 10, 15 and 20 different staple sequences that means a reduction in number of different strands of 15-, 10- and 7.5-fold, respectively. For comparison, the standard DNA origami scaffold M13mp18 contains only 2% sequence redundancy by this measure. The staple lengths ranged from 38–77 bases and a crossover density of 240 crossovers per 1,000 nucleotides. We successfully folded all three versions as can be seen by the transmission electron micrograph class averages.

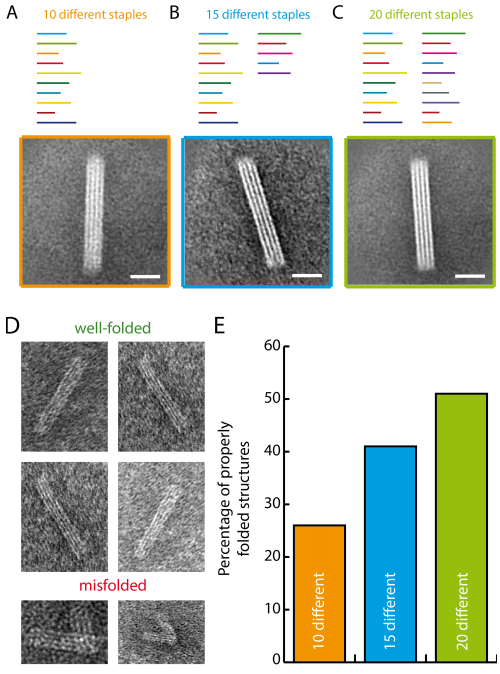


Figure 2. DNA nanostructures folded with custom scaffolds using 10, 15 or 20 unique staple sequences binding in multiple template locations. TEM class averages of 24-helix bundles designed with (A) 10 unique staples that each bind 10±2 template locations, (B) 15 unique staples that each bind in 7 ± 1 template locations and (C) 20 unique staples that each bind 5 ± 1 template locations. Scale bars: 20 nm. (D) TEM micrographs of representative well-folded (top) and misfolded (bottom) 24-helix bundle particles. (E) Absolute folding yields for designs with 10 (orange), 15 (blue) and 20 (green) unique staple sequences, as determined by gel electrophoresis and by manual counting of 142–355 particles in electron micrographs for each design.

SIGNIFICANCE: A crucial challenge for future industrial-scale applications of DNA origami is the difficulty and high cost of manufacturing large amounts of DNA nanodevices. In the past decade, DNA-based nanodevices have been created from hundreds of unique components. Typically these components include hundreds of DNA oligonucleotides 20-50 bases in length, and one or more virus-derived "scaffold" 7-8kb in length). Our method shows that a design strategy of sequence reuse is viable in a 1-pot folding reaction. Future designs may benefit from significant reductions in the number of unique DNA oligonucleotide sequences required for successful folding.

2. We created a powerful new system to express custom ssDNA scaffolds.

To fabricate DNA origami nanostructures, researchers have long relied on the genome sequence of M13 phage, which cannot be easily customized. This constraint has held back progress in the field in general, and in our lab specifically because many of the applications we have in mind require custom DNA sequences.

As we reported in "**Construction of a novel phagemid to produce custom DNA origami scaffolds**. Parsa M Nafisi, Tural Aksel, Shawn M Douglas. *Synthetic Biology*: ysy015 2018," we developed a novel phagemid, pScaf, for creating highly customized ssDNA scaffolds with 10-kb lengths that can be produced at milligram-scale yields. We are now using this system to tackle many unanswered questions about how to optimize sequences for optimal folding. Moreover, we can now easily incorporate functional sequences, such as protein-binding sites, or incorporate adjacent thymine pairs to enable covalent UV cross-linking of DNA origami for improved stability.

Figure highlights from our publication:

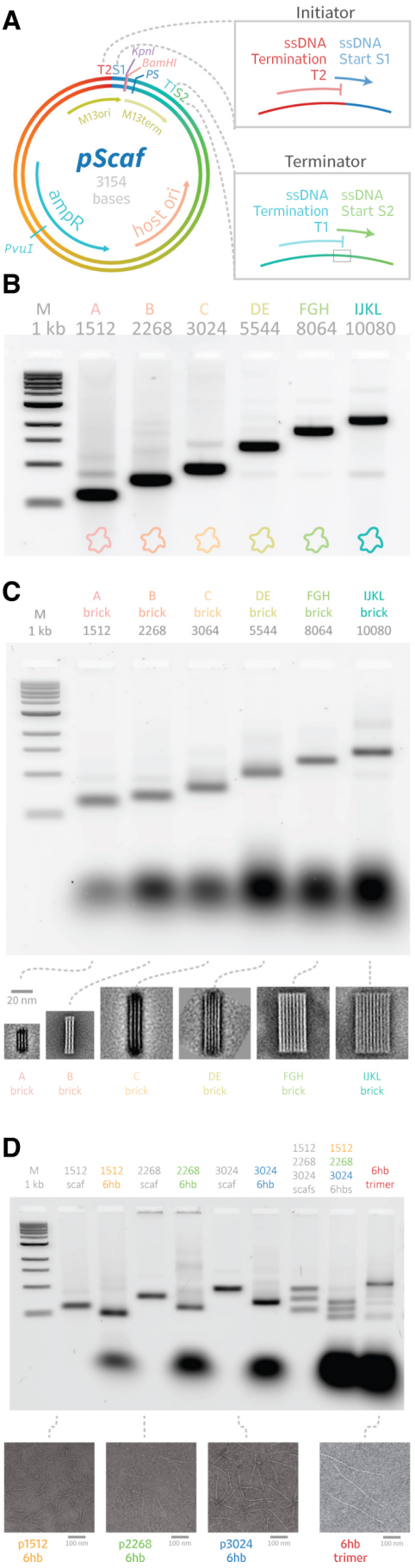
A. The pScaf phagemid enables an *E.coli* host to export a phage-like particle containing a custom ssDNA scaffold sequence. The key innovation was to use an M13 "origin" together and M13 "terminator" (a modified copy of the M13 origin with its initiation site disabled). Only the sequence in between these two sites is packaged and exported by the host strain.

B. Agarose gel electrophoresis shows custom scaffolds ranging from 1,512 to 10,080 nt in length.

C. We used custom scaffolds from (B) to fold DNA origami blocks designs of different sizes. Bottom: negative-stain TEM class averages of each block.

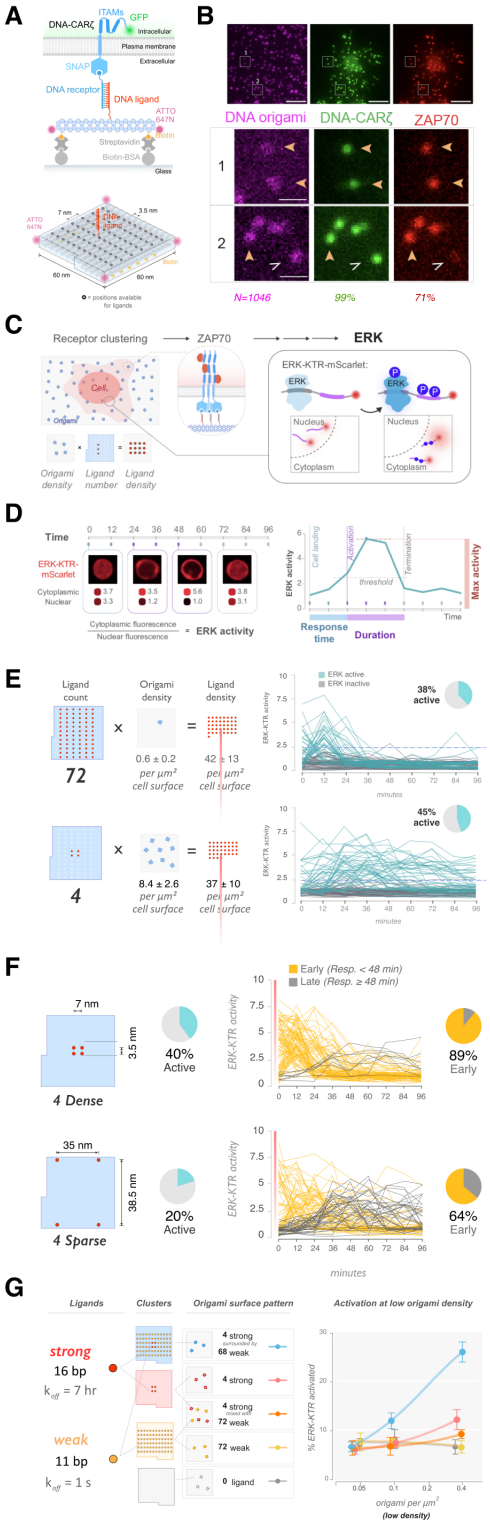
D. We demonstrated the one-put assembly of a single origami (last lane, 6hb trimer) from three separate scaffolds, paving the way for more complex assemblies in the future.

SIGNIFICANCE: Enables generation of new DNA scaffold sequences with which to use for building any origami structure. Unlike previous methods, our approach works with large scaffolds (10 kb) and scales up to milligram yields in shake flasks and to gram yields in bioreactors.



3. Our team has made significant progress in understanding the role of spatial organization in immune cell function.

We're all used to thinking about biology at the scale of molecules or angstroms, but there are all sorts of phenomena that happen at larger scales of 10 nanometers to 10 microns, a.k.a. the *mesoscale*. There are many longstanding questions about mesoscale organization and function of T cell receptors (TCR) at the earliest stages of T cell activation. Ron Vale's team created DNA-labeled T cell receptors or DNA-CARs to address these questions (Taylor *et al*. Cell 2017), but had little spatial control. We collaborated to build mesoscale DNA origami probe to gain precise control over ligand patterns, and reported our findings in "**DNA origami patterning of synthetic T cell receptors reveals spatial control of the sensitivity and kinetics of signal activation**. R Dong et al. (2021) PNAS. 118 (40) e2109057118" We have validated our approach and begun to probe spatial aspects of signaling that have long been inaccessible with other methods. Figure highlights:



A. Our DNA origami "pegboard" provides addressable, programmable control of a 6 x 12 grid of 72 "DNA ligands" that hybridize with the complementary "DNA receptor" on the synthetic T cell.

B. We confirmed that DNA origami successfully induces the first steps of T cell activation, namely clustering, read as GFP co-localization (99%) and ZAP-70 recruitment (71%, vs 6% in Taylor *et al.*).

C. We used a ERK-KTR biosensor to evaluate downstream signaling. Active ERK phosphorylates the sensor which then relocalizes to the cytoplasm.

D. We used the ERK-KTR biosensor to monitor origami-induced activation at single-cell resolution.

E. We found that cluster size is important. 72-ligand clusters induced a transient spike of ERK activity, while 4-ligand clusters led to durable activation. Origami concentrations are tuned to normalize ligand count per unit area of the cell surface.

F. Ligand spacing is important. Dense ligand spacing (3.5–7 nm) induced activation in 40% of cells, and 89% of those activate in under 48 min. Sparse ligand spacing (35–38.5 nm) only activates 20% of cells, and only 64% in under 48 min.

G. We built mixed-affinity origami pegboards and found that T cells were highly sensitive to 4 strong ligands (k_{off}=7hr) surrounded by 68 weak ligands (k_{off}=1s), which may have implications for future design of immunotherapies.

SIGNIFICANCE: Establishes a new paradigm for studying mesoscale receptor patterning in biology: DNA origami induces programmable clustering of DNA-labeled receptors on live cells; adaptable to many other systems. Addresses longstanding questions about organization and role of receptor clustering in T cell activation.

4. We applied our DNA origami spatial probes to study phagocytosis.

Macrophages destroy pathogens and diseased cells through Fcγ receptor (FcγR)-driven phagocytosis of antibody-opsonized targets. Phagocytosis requires activation of multiple FcγRs, but the mechanism controlling the threshold for response is unclear. We developed a DNA origami-based engulfment system that allows precise nanoscale control of the number and spacing of ligands, and reported it in "**Tight nanoscale clustering of Fcγ-receptors using DNA origami promotes phagocytosis**. N Kern et al. (2021) eLife 68311" When the number of ligands remains constant, reducing ligand spacing from 17.5 nm to 7 nm potently enhances engulfment, primarily by increasing efficiency of the engulfment-initiation process. Tighter ligand clustering increases receptor phosphorylation, as well as proximal downstream signals. Our results suggest that macrophages use information about local ligand densities to make critical engulfment decisions, which has implications for the mechanism of antibody-mediated phagocytosis and the design of immunotherapies.

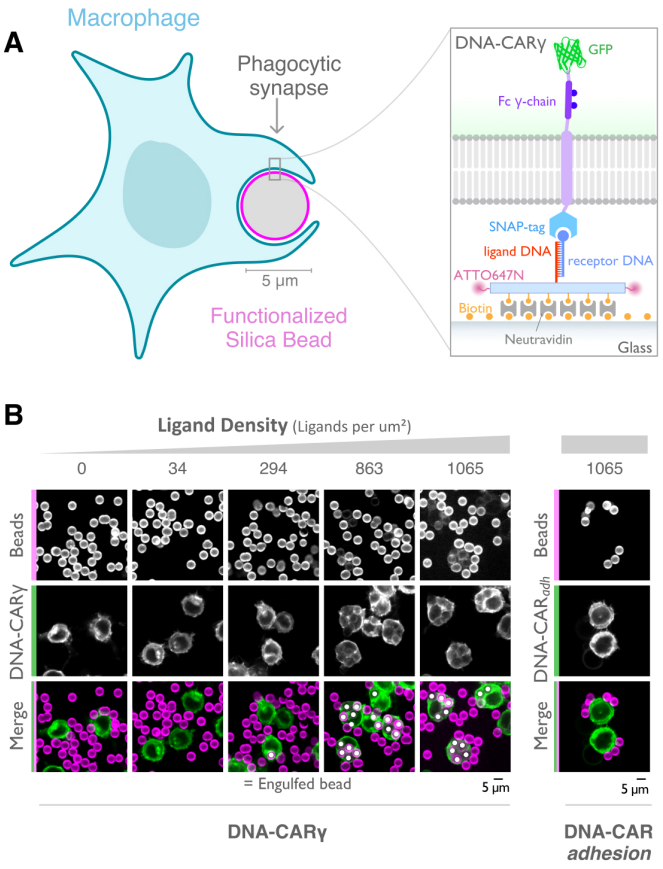


Figure highlights:

A. We adapted our DNA origami to an established "bead engulfment" assay by coating silica beads with origami pegboards. The macrophage cell has been modified to express synthetic Fc γ-chain that display an extracellular SNAP-tag linked to a ssDNA receptor.

B. The average number of beads engulfed per macrophage (white dots) was assessed by confocal microscopy.

C. We found that the spatial arrangement of ligands within nanoclusters regulates engulfment. Dense clusters (3.5–7nm spacing) induced 66% successful engulfment vs. 24% success by sparse clusters (35–38.5 nm spacing).

D. Tightly spaced ligand clusters promoted more tSH2-Syk recruitment, indicating the spacing plays an important role in the earliest downstream signaling events following FcγR binding to an engulfment substrate.

SIGNIFICANCE: First study of macrophage Fcγ-receptor clustering with sub-10-nm spatial resolution. This is the second application of our DNA origami platform enabling mesoscale receptor patterning on live cells; demonstrates power and generality of our approach.