Request for Information (RFI)

DARPA-SN-25-46

Physics-based control over *de novo* synthesis of DNA or RNA

Responses due March 20th, 2025, 4:00 PM ET

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URL: https://www.darpa.mil/research#research-opportunities

The Defense Advanced Research Projects Agency (DARPA) Biological Technologies Office (BTO) seeks to gain a better understanding of biotechnological advancements and gaps that could contribute to the ability to synthesize *de novo* DNA and RNA sequences *in vivo*. The goal of this RFI is to gather information on the possibility and challenges in developing a platform for *in vivo* (i.e., in a living cell) synthesis of DNA/RNA, where the nucleic acid sequence is precisely defined by patterns of physical stimulation (i.e., optical, mechanical, sound, electrical, thermal, etc.) rather than using a DNA/RNA template strand. The ultimate aim is for this template-free, *de novo* synthesis (i.e., capable of producing arbitrary sequences that may not be based on natural sequences) mechanism to produce DNA/RNA that can be translated into functional proteins (Fig 1). DARPA may elect to host a workshop predicated on subject of this RFI in Arlington, VA on May 1st, 2025, and a subset of respondents to this RFI may be invited to attend this workshop, in which case, their travel costs will be reimbursed.

Background

Synthetic DNA and RNA hold promise for providing solutions to global challenges involving rapid prototyping, genetic engineering, and biopharmaceuticals. However, traditional chemical and enzymatic methods for the *de novo* synthesis of DNA and RNA sequences for novel protein production are constrained by the size and complexity of the desired oligonucleotides, limited scalability, and environmental concerns[1,2]. Consequently, alternative biocatalytic approaches are needed. DARPA is looking to challenge the current state of the art technology and ask if it is possible to produce *de novo* DNA/RNA sequences *in vivo*

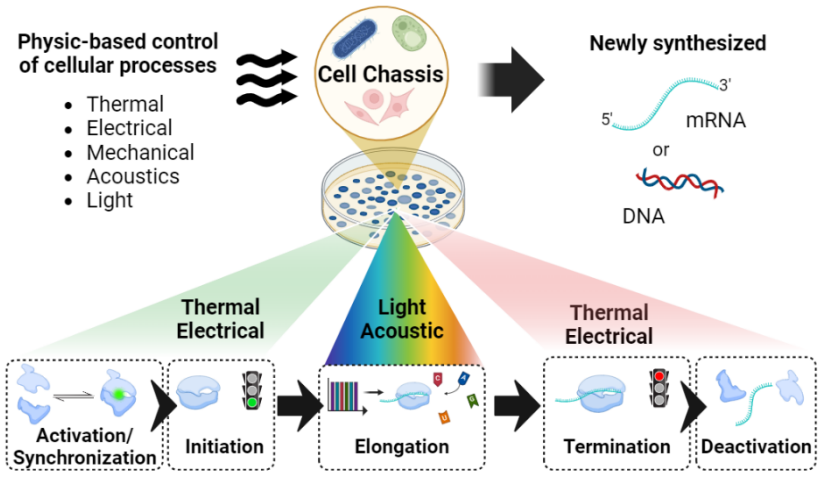

Figure 1. Schematic of *de novo* DNA or RNA synthesis *in vivo* via patterns of physical stimulation. Created in BioRender. DARPA BTO. (2024) https://BioRender.com/u13q901

via physics-based (i.e., light, mechanical, sound, electromagnetic, thermal, etc) control of cellular processes (Fig 1).

Strategic Approach

The process of synthesizing DNA or RNA requires the coordination of multiple processes and enzymes; this RFI specifically seeks to understand possible approaches to controlling the following processes *in vivo* with physics-based stimulations. For simplicity, we refer to the agglomeration of all processes and enzymes necessary to execute physics-based, template-free DNA/RNA synthesis merely as a 'synthase unit.'

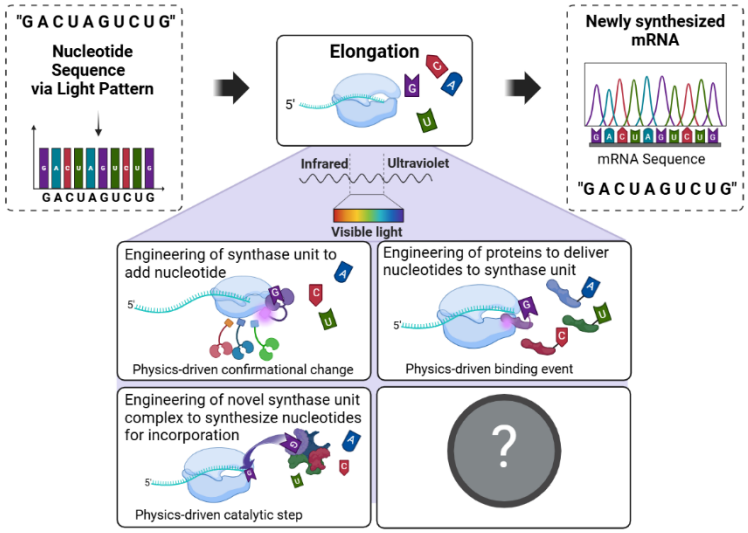

Figure 2. Potential strategies for light-controlled elongation of *de novo* DNA or RNA *in vivo*. Created in https://BioRender.com

1. **Initiation:**
    - What would be a possible strategy for activating and synchronizing the initiation process of DNA/ RNA synthesis *in vivo* across multiple *de novo* synthase units within cells and across populations of cells with a physical stimulus?

2. **Elongation:**
    - Describe a possible approach(s) to using temporal sequences of light or other patterns of physical stimuli to enable accurate control over nucleotide incorporation with single base-level precision during the elongation process in cells (Fig 2).
    - Terminal deoxynucleotidyl transferase (TdT) is commonly used to synthesize *de novo* DNA due to its ability to non-selectively add nucleotides to DNA[3,4]. However, where precise control over the addition of specific bases is necessary, particularly in response to physical stimuli, a more suitable approach may involve designing chimeric enzymes that can selectively bind nucleotides for elongation. For example, could this be achieved by engineering domains from dNTP-binding proteins[5,6], nucleoside diphosphate kinases[7,8], or RNA-specific ribonucleotidyl transferases[9,10]? Alternatively, would it be necessary to develop entirely new, non-natural enzymes that can facilitate the precise elongation of DNA or RNA strands? What would be the potential benefits or consequence of using a "non-traditional" enzyme for the elongation process?
    - To make a meaningful impact in fields such as synthetic biology, bio-manufacturing, environmental monitoring, and medicine, especially in the area of RNA therapeutics, the potential system must be capable of synthesizing nucleotide

sequences with real-time functionality. DARPA is interested in the possibilities and limitations of such a system, concerning the following:

- Kinetics: What would be the fastest elongation rate (base/min) possible to achieve with precision?
- Length: What would be an appropriate length threshold for a *de novo* DNA/RNA sequence with such a system?
- Accuracy: How would the system be optimized for maximum accuracy of nucleotide sequence?

3. **Termination:**
    - Describe methods for controlling transcription termination of nascent, *de novo* DNA and RNA sequences with a physical stimulus across a population of synthase units within and between cells.
    - What post-synthesis modification would need to be considered to ensure translation of the *de novo* sequence?
    - Ideally approaches will produce a functional protein and leave the cell intact with the ability to repeat this process with a different DNA or RNA sequence.

4. **State of Art:**
    - The current state of the art (SOA) methods (Table 1) for *de novo* DNA and RNA writing focus on combining chemical and enzymatic syntheses to attain sequence selection and high accuracy[1]. Additionally, *de novo* DNA/RNA synthesis-to-protein production involves several time-consuming steps, beyond just chemical or enzymatic DNA/RNA synthesis, such as plasmid construction and cell transformation (Table 2). What possible improvements over the current state of the art could be realized by an enzymatic platform that could convert patterns of physical stimuli into *de novo* DNA or RNA sequences *in vivo*?
    - From the perspective of biomanufacturing workflows (industrial and/or biopharmaceutical), agriculture, and therapeutic delivery to patients, would the ability to directly write DNA or RNA in a living cell using only patterns of physical stimuli to define the sequence create new efficiencies relative to current SOA?
    - Are there examples of how the improvements in efficiency over current SOA can be quantified in terms time, money, or safety? Examples ranging from cellular engineering/strain development to the supply chain and logistics of product deliver are of interest.

Table 1. State of the art DNA and RNA synthesis.

Nucleotide Sequence	Method	Template	Rate	Length (bp)	Accuracy/Efficiency	Ref
DNA	Phosphoramidite	Template-free	20-45 min/base	10 - 200	95.1% 60.6% 36.7%	[11]

DNA	Taq PCR	Template	15-60 sec/kb	5 kb	Error rate 1 in $4.3*10^5$	12,13
DNA	High fidelity polymerase	Template	15-30 sec/kb	Up to 40 kb with optimization	Error rate 1 in 2-$3*10^6$	12,13
DNA	DNA Printer	Template-free	10-20 min/base	120	~78% yield for 50 nt oligo	14,15
RNA	Phosphoramidite	Template-free	20-30 min/base	40	~37%	16,17
RNA	Polymerase (T7)	Template	~5 sec/kb	Up to 27 kb	Error rate 1 in 2×10^4	18–20

Table 2. Delivery of DNA/RNA into cells.

Method	Size Limit (kb)	Efficiency	Stability	Time to validate success	Cell Types	Ref
Electroporation	• 10 -15 kb • Bacterial artificial chromosome with loss of viability and efficiency	• ~10^9 cfu/ug pUC19 • 20% in hard to transfect cells • 10^6 cfu/ug	Transient or stable	• Transient: hours to days • Stable: weeks	Bacterial, yeast, plant, eukaryotic	21–24
Lipid transfection	• 15-20 kb plasmid	• 60-90%	Transient or stable	• Transient: 1-3 days • Stable: 2-3 weeks	Eukaryotic	25–27
Viral transduction	• 10 kb	• Wide variation depending on vector & cell type	Transient or stable	• Transient: 1-3 days	Mammalian and insect	28,29
Bacterial transformation (chemically competent cells)	• 10 kb	• 10^4 to 10^8 cfu/ug DNA	Stable with antibiotic pressure	• 1 day	Bacteria	30,31

5. **Biosecurity:**
    - Advances in genomic technologies allow for a deeper understanding of DNA and RNA, but they also catalyze the need for standardized specification and procedures to ensure results are consistent, reliable, and safe. With these rapid advancements, new security vulnerabilities emerge that require novel enabling technologies for the

identification and prevention of potential misuse. What should biomonitoring programs aimed at developing guidelines and policy consider when trying to reduce and monitor the risk of a capability that would enable synthetic DNA and RNA production inside a cell line capable of translating those genetic instructions?

- Current frameworks, such as the 2024 Office of Science and Technology Policy (OSTP) Framework for Nucleic Acid Synthesis Screening and the 2023 Health[32] and Human Services (HHS) Guidance[33], aim to develop guardrails for safeguarding nucleic acid synthesis prior to procurement and use in biological systems. What are some of the biosecurity vulnerabilities arising from engineered i*n vivo* nucleic acid synthesis, that are not addressed in the current frameworks?
    - While responses focused on cyberbiosecurity mitigations to secure software and hardware systems that would be necessary to deliver physical stimuli are welcome, approaches to securing Generative Optogenetic systems (i.e., cells expressing synthase units) at the level of biology are highly encouraged. Are there examples of additional technologies that could be developed to increase the inherent security of cells expressing Generative Optogenetic synthase units?

Programmatic Approach

Additionally, we hope to receive concise input that would help us structure a feasible and successful program that triggers development of innovative technologies with strategic capabilities.

1. **Schedule:** Appropriate milestones within the program and reasonable timeframes for the completion of these milestone.
    - Other suggestions for how a program could be structured to best allow for solutions to be successful
2. **Cost:** Minimal dollar amount for the award, for solutions at different stages of maturity (e.g., ideas for promising, novel approaches, preliminary *in vitro* and *in vivo* data).

NO CLASSIFIED INFORMATION SHOULD BE INCLUDED IN THE RFI RESPONSE.
It is the submitter's responsibility to clearly label proprietary information contained in the RFI response. DARPA will not disclose information labeled as proprietary.

Responses can address any one or multiple of these considerations. RFI responses should also provide a rough estimate of achievable performance and indicate specific issues to be addressed, such as those listed earlier.

DARPA invites responses from all capable and qualified sources including, but not limited to, universities, University-Affiliated Research Centers, U.S. Government laboratories, Federally Funded Research and Development Centers, and private or public companies.

Workshop

DARPA is considering hosting an invitational workshop on May 1st, 2025 in Arlington, VA with the purpose of reviewing and discussing current and future research relevant to this RFI. DARPA may provide travel reimbursement to a subset of invited responders to RFI for participation in the workshop. Information discussed at this workshop may assist in the formulation of possible future areas of research with the objective of template-free, *de novo* synthesis mechanism to produce DNA/RNA that can be translated into functional proteins. If DARPA determines to proceed with workshop invitations to RFI respondents, invitations will be sent out by April 1st, 2025.

Format

Each submission should be typed in 12-point, single-spaced font on 8.5- by 11-inch pages, with 1-inch margins. All submissions must be electronic, adhere to the content formatting described below, and use one of the following file formats: Adobe PDF or Microsoft Word.

Responses should include:

1- Cover Page (one page)
   a. Title
   b. Organization
   c. Responder's technical and administrative points of contact (names, addresses, phone numbers, fax numbers, and email addresses)

2- Technical Response to this RFI should be written narratives that provide details and insight to the questions posed, as this will enable an understanding of the current state of the art, identify key limitations and challenges, and explore the possibilities and potential applications of the technology. The information gathered through this RFI will be used to inform the development of a future program.

   H1. What would be the objective of the research? Articulate with no jargon.

   H2. What is the state-of-the-art and its limits?

   H3. What is new about the listed approaches and why would they succeed?

   H4. Who cares? If successful, what difference will it make?

   H5. What are the risks?

   H6. How much will it cost?

   H7. How long will it take?

   H8. What are the mid-term and final metrics to determine success?

3- References (no limit)

4- Appendix: Responder's relevant experience and expertise including citations of their relevant peer-reviewed literature. Relevant published papers (limit 3).

Submission

All technical and administrative correspondence, questions regarding this announcement, how to respond to this RFI, and submissions themselves should be sent to DARPA-SN-25-46@darpa.mil. Please refer to "Generative Optogenetics RFI" in all correspondence. Emails sent directly to the Program Manager(s) may result in a delayed response or no response.

Disclaimers and Important Notes

This is an RFI issued solely for information and new program planning purposes; it does not constitute a formal solicitation for proposals. This RFI is not tied to any funding for research and submission of proposals is discouraged as they will not be reviewed. In accordance with FAR 15.201(e), responses to this notice are not offers and cannot be accepted by the Government to form a binding contract. Submission is voluntary and is not required to propose to a subsequent Broad Agency Announcement (BAA) (if any) or other research solicitation (if any) on this topic. DARPA will NOT provide reimbursement for costs incurred in responding to this RFI. NO CLASSIFIED INFORMATION SHOULD BE INCLUDED IN THE RFI RESPONSE. It is the submitter's responsibility to clearly define to the Government what is considered proprietary data. Any proprietary information should be clearly labeled as "proprietary." Respondents are advised that DARPA is under no obligation to acknowledge receipt of the information received or provide feedback to respondents with respect to any information submitted under this RFI.

Reference

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