Special Notice (SN) DARPA-SN-26-19: Generative Optogenetics (GO) Proposers Workshop

DATE: January 7, 2026
LOCATION: Greater DC area (venue location TBD)
REGISTRATION WEBSITE: https://events.sa-meetings.com/GOProposersWorkshop/
REGISTRATION DEADLINE: **Monday, December 29, 2025, at 4:00 PM EST** or when capacity is reached, whichever comes first.
TECHNICAL POC: Dr. Matthew Pava, DARPA/BTO

The Biological Technologies Office (BTO) of the Defense Advanced Research Projects Agency (DARPA) is hosting a Proposers Workshop in support of an anticipated Program Solicitation (PS) for the Generative Optogenetics (GO) program. The Proposers Workshop will be held on Wednesday, January 7, 2026, in the Greater DC area (specific venue location to be announced on the registration website). **Advance registration is required.** Registrants will have the opportunity to provide teaming profiles and give a lightening talk. There are a limited number of timeslots for lightening talks, so they must be scheduled upon registration on a first-come, first-served basis. Note, all times listed in this announcement and on the registration website are Eastern Time.

The goals of the Proposers Workshop are to:

1. Support ideation of approaches to address the goals of the GO program
2. Increase the efficiency of proposal refinement and preparation; and
3. Encourage and promote teaming arrangements among organizations with the expertise, research facilities, and capabilities needed to execute research and development responsive to GO program goals.

DARPA anticipates releasing the GO Program Solicitation (PS) in December 2025. When released, the PS will be available on https://sam.gov/. *Draft (non-final) excerpts of the GO PS are included at the end of this Special Notice.* To maximize the pool of innovative proposal concepts, DARPA strongly encourages participation in the Proposers workshop and related solicitations by non-traditional performers, including small and medium-sized businesses, as well as academic and research institutions, including first-time government contractors. While attendance at the workshop is not mandatory for submitting a proposal or being selected for funding, it provides valuable insights and guidance. All information shared during the Proposers Workshop will be accessible on the GO Program page:

https://www.darpa.mil/research/programs/go

DARPA will publish a technical video on the GO Program page that provides a deep dive into the GO program. The video will cover everything from the program's core structure and overarching goals to the enabling technologies it leverages and the anticipated outcomes it aims to achieve. Individuals interested in attending the Proposers Workshop are highly encouraged to review this video in advance to make maximal use of the available time.

It is expected that GO will require strong teaming efforts to successfully innovate and integrate critical technologies necessary to meet the metrics and associated timelines of the GO program. The Proposers Workshop will include a brief introduction by government personnel followed by lightning talks from potential proposers wishing to highlight technical capabilities and promote teaming. DARPA will collect participant questions throughout the event and publish answers to a publicly available FAQ document.

Proposers Workshop Participation:

This Special Notice for Proposers Workshop includes opportunities for prospective proposers to identify team members:

1. *Lightning Talks.* Interested attendees are invited to submit one (1) MS Power Point or Adobe PDF slide that summarizes their interests and capabilities using the template provided (**Attachment 1**) with this notice and on the registration website. These will be presented during 5-minute "lightning talks" at Proposers Workshop. Submitted presentations consisting of multiple slides or a single slide with multiple layers will not be granted a time slot. Lightning talk submissions (no more than one per organization) will be accepted on a first-come, first-served basis, until the maximum possible number of submissions (given time constraints) is reached. Interested attendees must identify their intention to submit the lightning talk during registration. Due to limited availability, DARPA does not guarantee that these requests will be fulfilled.

2. *Poster Session.* Attendees may also choose to present a poster describing their research interests or expertise that will be available for viewing and interaction during a portion of the Workshop. Those interested may submit both a lightning talk and a poster. Posters must adhere to the following guidelines:
   - Posters should be sized exactly 24" x 36" (either horizontal or vertical orientation).
   - Presenters are responsible for printing their own poster.
   - The Workshop will provide easels with foam boards and clip/pins to mount posters to the boards.

3. *Teaming Profiles.* Interested parties are also invited to submit a one- to two-page 'teaming profile' describing technical competencies, unique facilities and other capabilities, as they relate to the program, and desired technical/other competencies sought from other potential team partners. The profile should conform to the template provided with this notice and on the registration website. Further details will be provided on the registration website, but teaming profiles should include, at a minimum:
   - Contact information, to include name, organization, email, telephone number, mailing address, and website;
   - Brief description of the proposer's technical competencies and relevant facilities;
   - Desired technical competencies and facilities from other potential team members, if applicable.

Profiles that do not use the provided word template (**Attachment 2**) and/or exceed the two-page limit will not be accepted. Information contained in teaming profiles shall be publicly releasable – profile submitters consent to distribution amongst event registrants. After Proposers Workshop, the teaming profiles will be sent via e-mail to all registrants. Specific content, communications, networking, and team formation are the sole responsibility of participants. Neither DARPA nor the DoD endorses any participating organization, nor does DARPA or DoD exercise any responsibility for improper dissemination of the teaming profiles.

All conforming lightning talks, posters, and teaming profiles must be emailed to GO@darpa.mil no later than **4:00 PM EST on January 5, 2026**. Specific content, communications, networking, and team formation are the sole responsibility of the participants.

Registration Information:

PLEASE NOTE: Registration closes Monday, December 29, 2025, at 4:00 PM EST

Participants must register in advance through the registration website. Registration is free but limited by the venue capacity, so early registration is recommended. Interested parties are encouraged to coordinate attendance within their organizations prior to registration. Attendance is limited to no more than three representatives per division/department, with a maximum of five attendees from any one organization.

Interested parties are highly encouraged to send their technical PIs as well as a Contract representative. DARPA will have a dedicated 'ask me anything' table related to the associated contractual and grant-related requirements and intricacies of working with DARPA on the GO program. Individuals who are unable to register because the deadline has occurred or capacity has been reached will be added to a waitlist. Slots that remain open after registration closes or that become available due to cancellations will be filled on a first-come, first-served basis from the waitlist. An online registration form and various other meeting details can be found at the registration website, https://events.sa-meetings.com/GOProposersWorkshop/.

All U.S. Permanent Residents and Foreign Nationals must submit a DARPA Form 60, except foreign government personnel who must submit only an Official Visit Request completed by their respective Embassy based in Washington, DC. All forms must be submitted no later than **4:00 PM EST on December 29, 2025**. Form 60 submission instructions are provided on the registration website and in the registration confirmation email. Contact your Embassy staff for assistance in submitting the Official Visit Request.

Further administrative questions should be addressed to GO@darpa.mil. Please refer to the GO Proposers Workshop (DARPA-SN-26-19) in all correspondence. This announcement is not a request for abstracts or proposals; any sent will be disregarded.

DARPA hosts Proposers Workshops to provide potential performers with a venue for interacting to facilitate teaming and to increase efficiency in proposal preparation and evaluation. Therefore, Proposers Workshops are open only to potential proposers who have registered.

This notice is issued solely for information and potential new program planning purposes; the SN does not constitute a formal solicitation for proposals or proposal abstracts. 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 subsequent Broad Agency Announcements (if any) or research solicitations (if any) on this topic. DARPA will not provide reimbursement for costs incurred in responding to this SN. 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 SN.

NO CLASSIFIED INFORMATION SHOULD BE INCLUDED IN THE SN RESPONSE.

DRAFT PROGRAM INFORMATION:

The program information provided below on pages 4-18 is a DRAFT excerpt from the Generative Optogenetics program solicitation. To that end, the information listed below (and throughout) is a draft **and subject to change** when the final solicitation is posted. The details outlined in the official program solicitation will take precedence over any information contained in this special notice.

Program Background:

Synthetic DNA and RNA are essential molecules for technologies that address critical global and national security challenges related to resilient supply chains, advanced materials manufacturing, agriculture, and human health. However, traditional methods for the *de novo* synthesis of DNA and RNA sequences are constrained by the size and complexity of the desired oligonucleotides, limited scalability, and environmental concerns. Current methods for nucleic acid synthesis fall into two categories: catalyzed by proteins (i.e., enzymatic) or synthetic chemistry. Naturally, cells replicate copies of their genomes and produce RNA via enzymatic processes, achieving remarkable speed and accuracy with error rates as low as 1 in 10^{10}. This fidelity is driven by mechanisms such as template-directed polymerization, proofreading freshly synthesized nucleic acids, and DNA mismatch repair. However, none of these processes are capable of *de novo* synthesis because they all require a nucleic acid template.

For template-free production of nucleic acids, chemical phosphoramidite synthesis is the gold standard. Considering the entirety of the phosphoramidite process, DNA and RNA synthesis rates can take 20–45 min per base and error rate of 0.01 per base. Phosphoramidite synthesis is constrained by sequence length (200 bp for DNA, 40 b for RNA) and requires extensive downstream processing to make nucleic acids biologically compatible. Recent advancements in cell-free enzymatic synthesis have improved *de novo* synthesis rates to 1.3–10 minutes per base, with error rates ranging from 0.01 to 0.2 per base. However, enzymatic methods remain limited by fragment size and centralized production, requiring additional steps for assembly and delivery into cells. Furthermore, while approximately 70% of human protein-coding RNAs are ≤3 kb in length, current state-of-the-art methods cannot achieve single-shot synthesis of such sequences. Instead, substantial downstream efforts are required to assemble shorter fragments into longer protein-coding sequences, a process that must occur outside the cell. This process can take anywhere between 10 days to over a month. **No existing technology enables massless information transfer to relay genetic instructions to living cells. All current approaches require some mechanism predicated on moving matter that encodes the genetic information, typically DNA or RNA nucleic acids, across biological barriers like a cell wall/membrane.**

Generative Optogenetics (GO) program aims to create a molecular machine that can be expressed in living cells and provide a mechanism for transducing genetic information transmitted masslessly via optical signals into the nucleic acid sequences (DNA and/or RNA), which are the native information storage for all known life. Such a capability will create a direct interface between computers used to design genetic sequences and living cells that operate on those sequences. Technology developed on GO will enable unprecedented control over cellular

behavior by facilitating genetic programing with single-cell spatial resolution, temporal precision to deliver different messages to a cell sequentially, and remote, scalable dissemination of genetic instructions.

At its core, GO addresses the high-risk challenge of developing a novel, open-ended genetic control platform that functions *in vivo* (i.e., within a living cell) to accelerate the transmittal of genes to living systems. If successful, this technology is anticipated to unlock a foundational capability with ramifications for medicine, agriculture, and manufacturing, while diminishing reliance on brittle supply networks that become untenable for long distance operations, like extended human spaceflight. GO is solely focused on building a proof-of-concept to address this high-risk challenge, but performers that meet the program's ambitious goals will pave the way for broadly transformative applications.

Acquisition Strategy:

Abstract submission deadline is anticipated to be January 16, 2026, 4:00PM (Eastern Time). Abstracts will be reviewed by the Government; if selected, the proposer will be asked to brief an Oral Presentation Package (OPP). OPPs will be reviewed by the Government, and if selected, may result in a Phase 1 award of an Other Transaction and eligibility to participate in future Phases of the program.

DARPA encourages solutions from all responsible sources capable of satisfying the Government's needs, including large and small businesses, *nontraditional defense contractors* as defined in 10 U.S.C. § 3014, and *research institutions* as defined in 15 U.S.C. § 638.

To facilitate this objective, the Government will use the following acquisition process for GO:

1. **Abstracts**: Through the forthcoming program solicitation, the Government will require proposers to submit 5-page Abstracts as the first step in the acquisition process. The Government will review all submitted Abstracts for technical comprehension and ability. Selected proposers will be invited to brief their OPP to the Government. The Government will review the submitted Abstracts to gain a high-level understanding of each proposing team's strategy to develop a prototype molecular machine capable of functioning in a living cell to transduce genetic information encoded in optical signals into protein coding nucleic acid sequences. Predicated on the submitted abstracts, the Government will decide to invite a subset of proposing teams to brief OPPs in an Oral Presentation.
2. **Oral Presentations**: Upon the Government's request, proposers will have the opportunity to present their OPP to the DARPA program team. The Government will evaluate all OPPs and anticipates that selected performers will be given fixed-price Other Transactions (OT) for Prototypes awards to address Phase 1 goals/metrics over a 12-month period of performance. Oral presentations of the OPP will afford the Government an opportunity to ask clarifying questions of the briefing teams. The OPP in its entirety, including the briefing itself, will establish the basis for selection decisions for Phase 1, and consequently, far more detail about each team's project plan is expected in the OPP versus the Abstracts. While awards made following the OPP will be for Phase 1 only, the content of the OPP should describe each team's overall plan to develop their molecular

machine, including planned Phase 2 effort, with particular emphasis placed on how the proposed Phase 1 effort will de-risk and refine the strategy for Phase 2. It is not expected that Phase 2 plans will be finalized at this time, but proposing teams should articulate a reasonably detailed draft, including a design and test plan for their proposed mechanism of transducing genetic information transmitted masslessly via optical signals into nucleic acid sequences. The draft Phase 2 plan should align to the goals of the program for demonstrating this transduction mechanism in a living cell. Based on this draft plan for Phase 2, the OPP must describe a set of clear, finalized Phase 1 tasks, and it must justify how these tasks will de-risk and inform the finalization of the Phase 2 plan by month 9 after award.

3. **Phase 1 (12 months):** Performers will refine and de-risk their work plan for Phase 2. The revised work plan will be provided to DARPA in a written form (i.e. a Task Description Document, TDD, that may be included in the OT agreement) as well as in an oral presentation. All Phase 1 performers will present their final revised Phase 2 plans during the Concept Design Review (CoDR), scheduled approximately ~9 months after the Phase 1 agreement award.
4. **Phase 2 (30 months):** Subject to Phase 1 performance, assessed Phase 2 plans, and available funds, DARPA may negotiate Phase 2 using the existing OT agreement from Phase 1. Performers advancing to this phase will execute their technical plan for developing a prototype molecular machine capable of functioning in a living cell to transduce genetic information encoded in optical signals into protein coding nucleic acid sequences.

Program Description/Scope:

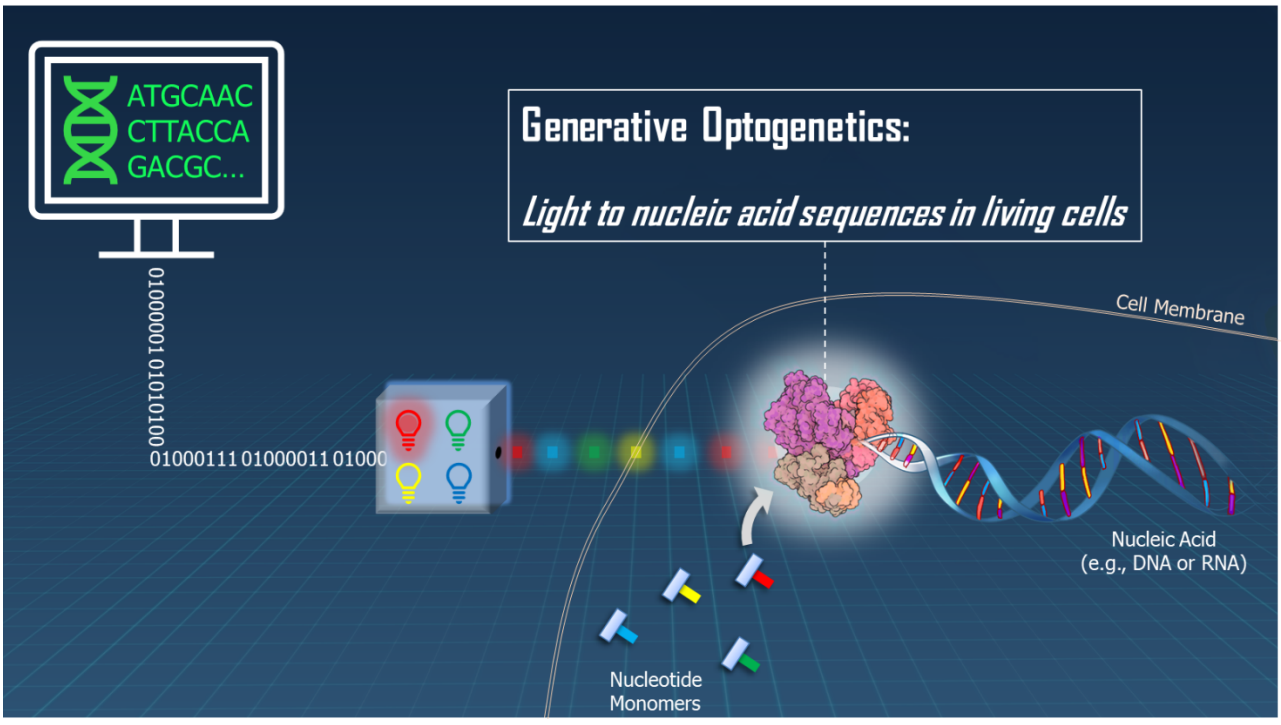

Figure 1. Overview of the GO program. The goal of the program is to develop a protein complex that can be expressed in living cells to transduce genetic information transmitted to the cell in the form of an optical signal (e.g., sequence of light pulses) into a nucleic acid sequence that the cellular host can operate on via transcription and/or translation mechanisms native to that cellular host.

Overall Scope of the GO Program

The DARPA GO program aims to develop a protein complex, referred to here as a nucleic acid compiler (NAC), that can be expressed within living cells to allow an end user to program genetic instructions into those cells, template-free, using nothing but light to transfer the genetic information to the cells (Figure 1). The central challenge of developing the NAC involves integrating protein domains / subunits for precise optical-responsiveness (i.e., optogenetic domains), substrate binding, and enzymatic activity into a functional complex of proteins (i.e., a holoenzyme). While many of these domains have precedence as either engineered or naturally occurring proteins, these components must be made interoperable such that they can be combined into a NAC that responds rapidly and predictably to optical signals to synthesize long, accurate sequences that discernably alter cellular function as desired. Moreover, expression of the NAC itself must not be deleterious to host cell function or viability.

To develop the NAC, the GO program consists of two Research Objectives (ROs):

1. ***Research Objective 1 (RO1): De Novo* Synthesis – All responses to the GO Program Solicitation MUST address RO1** to describe how they will develop the core capability of the NAC for template-free DNA or RNA synthesis such that optical inputs dictate the sequence of the nucleic acid produced by the NAC in a living cell. Teams are strongly encouraged to focus their proposals on synthesis of either DNA or RNA, although this selection is not a strict requirement. Responses to RO1 must clearly describe how they intend to address three key challenges: engineering constituent protein domains, integration of domains into the NAC, and the stability of the NAC-nucleic acid complexes.
2. ***Research Objective 2 (RO2): Error Mitigation* – OPTIONAL, responses to the GO Program Solicitation may elect to address RO2 in addition to RO1** such that their NAC designs enable high-fidelity synthesis by incorporating molecular detection mechanisms, which identify and filter out sequence errors. Some applications of GO technology will necessitate NACs capable of synthesizing longer sequences, and it is anticipated that increasing the length of the sequence will increase the likelihood that the synthetic sequences contain errors. To this end, the goal of RO2 is to explore the tradeoffs inherent to designing a NAC that meets more stringent error tolerance.

All GO performers MUST, at minimum, address RO1. Teams responding to this solicitation may elect to include plans to address RO2 in conjunction with RO1 in the same proposal. Because RO2 cannot be pursued as a standalone effort, any response that addresses only RO2 without also addressing RO1 will be deemed non-compliant and will be excluded from review. To this end, abstracts and oral presentations addressing RO2 MUST clearly indicate which elements of the approach pertain to the base NAC (RO1) and which elements represent additional error mitigation mechanisms (RO2).

Success on the GO program will necessitate the integration of multiple discrete protein functions into a holistic complex that facilitates scalable, massless information transfer into living cells. There are large extant bodies of research on the sequence, structure, and function of: optogenetic domains, nucleic acid polymerases/transferases, accessory proteins that stabilize nucleic acids to regulate formation of secondary structures as well as their association

with polymerases, and nucleotide binding domains. Proposing teams must justify how they plan to use this existing basis of knowledge to guide development of NAC, and specifically, they must include discussion about primary and secondary approaches to mitigate risk associated with integration of multiple, highly disparate domains into a functional NAC.

Along these lines, development of the NAC likely will require engineering multiple protein domains to integrate them into a holoenzyme capable of precise, multiplexed optical control over nucleotide incorporation. GO Performers may need to expand or adapt the available corpus of protein domains to integrate them into the NAC. For example, it may be necessary to refine optogenetic domains such that they can be multiplexed to control incorporation of four different nucleotide substrates. While the program is purposefully designed to allow time for domain design and optimization, these activities are not the main focus of the program. Consequently, successful GO performers must constrain iterative development of these protein domains to the absolute minimum necessary for developing a NAC that is fully functional in a living cell. To this end, the GO program is not an appropriate funding mechanism to support approaches that include bioprospecting to identify and characterize wholly new domains or proteins from natural systems. Similarly, exploratory work focused on phenomenological or mechanistic characterization of novel natural proteins that respond to light or other physical signals is specifically excluded. Both abstracts and oral presentations in response to this solicitation must address how the design or refinement of protein domains will be integrated into the NAC. Therefore, all respondents must justify both their plans for protein domain design/optimization and their use of existing domain structure/sequences in the context of the overall strategy for integration into the NAC.

To address the challenge of integrating protein domains into the NAC, proposers are strongly encouraged to leverage advancements in computational design tools for protein engineering, including generative AI approaches. These resources may serve as effective tools for optimizing substrate binding sites, allosteric interactions, and domain integration, and, as appropriate to a team's technical approach, proposers should feel free to combine them with rationale design methods that rely more heavily on empirical structural biology datasets. It may be necessary to tailor or combine existing computational tools to design the NAC, and in this case, proposing teams must clearly and succinctly describe the necessary development work and justify why the resulting functionality will be essential to the team's success on GO.

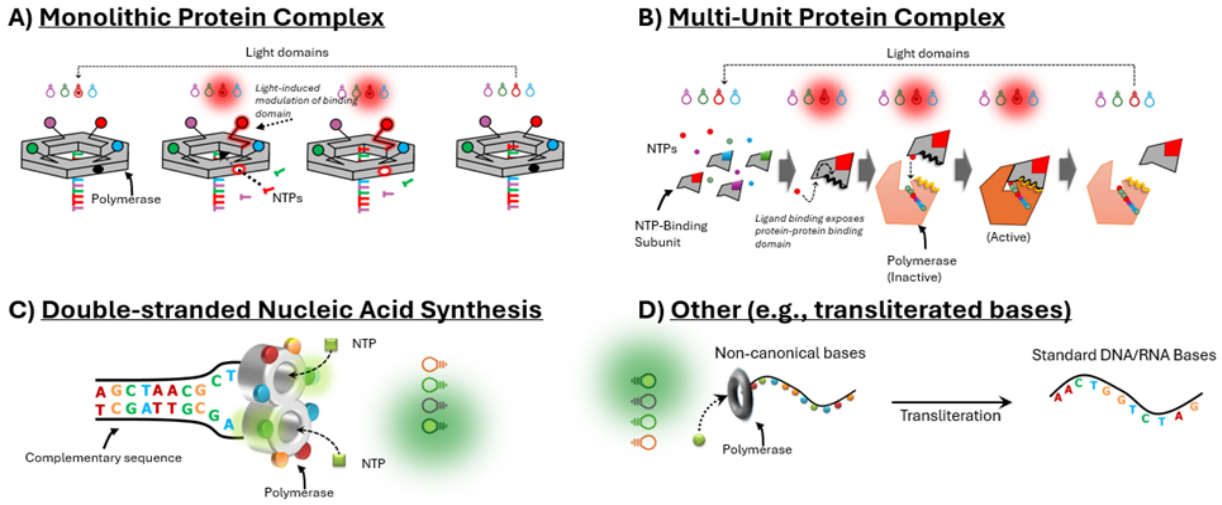

Figure 2. A non-exhaustive, non-mutually exclusive subset of possible high-level designs for a NAC.

There are many possible designs for a NAC that can function in a living cell (Figure 2), and different designs may be better suited for some cellular environments over others. GO RO1 performers can pursue any design they wish provided the following are true:

1. The resulting NAC can function inside a living cell to synthesize nucleic acid sequences with light (i.e., an optical signal) as the sole source of information encoding the nucleic acid sequence.
2. The resulting system (i.e., the cell engineered to express the NAC) does not require any exogenous substrates (e.g., engineered, non-canonical nucleotides) beyond C, H, N, O, S, and P containing molecules that are typically included in standard broth or media. However, cell lines may be engineered to synthesize substrates for the NAC, if these are not produced by the cell's native metabolism. Abstracts and Oral Presentations should detail clear plans for this metabolic engineering.
3. The NAC and/or resulting cellular system must incorporate a plausible strategy to synchronously activate/deactivate a population of NACs expressed inside a cell, so they can initiate transduction of optical signals into the nucleic acid sequence at the same time. Abstracts and oral presentations must include a description of the mechanism for this synchronous activation as well as a means for deactivation to eliminate unwanted transduction. Proposers are strongly encouraged to consider mechanisms to prevent the unintended activation of the NAC, ensuring precise and controlled functionality.
4. The design of the NAC and cellular system expressing it are capable of synthesizing long, coding nucleic acid sequences that serve to modulate cellular function. Because the purpose of the NAC is to achieve single-shot synthesis of complete sequences, approaches that require *in vitro* steps to enable full synthesis of the desired construct (e.g., synthesis of smaller oligonucleotides within cells followed by lysis and ligation/annealing) are out of scope for GO.
5. The flow of genetic messages as they emerge from GO technologies must intersect with the cell's natural machinery for producing RNA and/or protein. Thus, approaches seeking to design entire systems that operate in parallel to the central dogma are out of scope. While the synthesis of nucleic acid sequences built from non-canonical bases is permissible provided the cell can produce its own non-conical nucleotide substrates, GO performers need to include a mechanism to convert these sequences into canonical DNA/RNA for transcription or translation by the host cells' existing enzymes. Abstracts and Oral presentations proposing this approach must clearly describe this conversion mechanism, and they must provide clear evidence that the mechanism and any associated enzymatic machinery are known.

Just as there is latitude in NAC designs for RO1, GO performers seeking to incorporate additional error mitigation mechanisms in response to RO2 are loosely constrained. There are a number of potential strategies to increase the accuracy of sequences transduced from light (Figure 3). Abstracts and Oral Presentations responding to RO2 must justify their proposed approach in concert with the overall design of their proposed NAC. While it is not required that GO performers make their technical approach to RO2 severable from their RO1, respondents to

this solicitation are encouraged to include a discussion in their oral presentations regarding if and how their RO1 and RO2 mechanisms could be separated. Furthermore, teams responding to RO2 must clearly articulate their planned mechanism for mitigating error in the absence of a chemical template and what tradeoffs in NAC performance are anticipated by exercising this mechanism. If multiple error mitigation mechanisms are proposed, the plan must detail their integration, tradeoffs when using all or some mechanisms, and an experimental approach to evaluate each mechanism's impact on sequence accuracy and yield in cells. RO2 performers may include additional non-invasive physical modalities beyond optical signals, but tasks to develop or discover completely novel mechanisms are out of scope for GO. In any case, teams proposing to incorporate an additional physical signal modality must justify their RO2 mechanism BOTH scientifically and in terms of plausibility within the context of a notional transitional/translational use case.

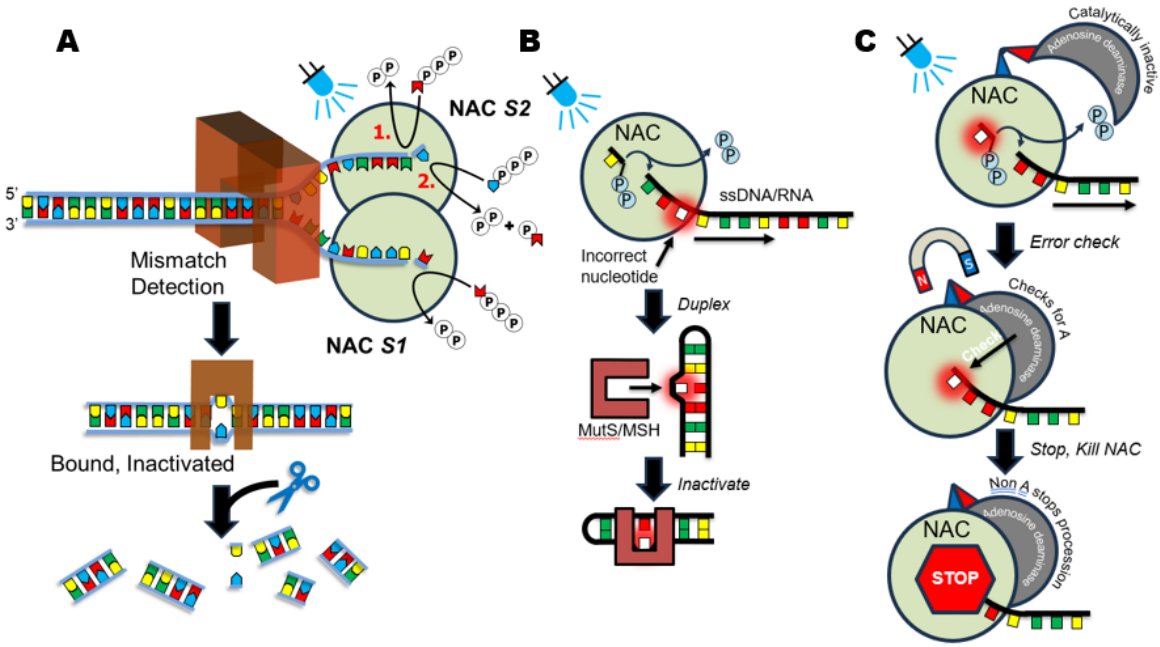

Figure 3. Notional non-exhaustive and non-mutually exclusive set of mechanisms for introducing error detection and mitigation to a NAC design. A, NACs synthesizing double-stranded molecules could include mechanisms based on base-pair mismatch detection; B, palindromic nucleotide sequences could be interleaved with mismatch detection inspired mechanisms; C, iterative toggling between a 'write' and 'error recognition' mode.

Over the course of development, it is expected that *in vitro* (i.e., cell-free) approaches will be necessary to demonstrate co-activation of optogenetic domains, opto-gated polymerases, and initial nucleic acid synthesis in highly controlled environments. In their Oral Presentations, teams responding to the GO PS should include justifications for both the need and appropriate selection of *in vitro* assays.

Ultimately, GO performers will develop and demonstrate a NAC capable of synthesizing full-length nucleic acid sequences between 3 and 6 kb *in vivo* (i.e., within a living cell). Since expression of the NAC in different cell types will, at minimum, affect the design of the NAC sequence (e.g., codon optimization), expression vectors, and/or means of genomic integration, GO performers will need to tailor their development strategy for their chosen cellular chassis that they will use to demonstrate *in vivo* functionality and performance. Additionally, the choice

of cellular chassis organism could affect the experimental approach to quantify NAC function *in vivo*. Therefore, abstracts and oral presentations should detail which chassis organisms or cell types are intended for *in vivo* demonstrations, and they should discuss how the proposed design and development of the NAC are appropriate for the intended host cell. GO performers are not limited to demonstrating the NAC in only one cell line, and using two or more cell lines for development of the NAC could facilitate more rapid development cycles. However, in their abstracts and oral presentations proposing teams should clearly state all cell lines they aim to use for development of the NAC, and in their oral presentations, teams proposing the use of more than one cell line should provide BOTH a scientific as well as technology transition-oriented and/or clinical translation-oriented rationale for each cell chassis they plan to test in. Additionally, oral presentations should also provide a brief high-level description of the planned approach for quantitatively assessing NAC performance *in vivo*.

GO performers may demonstrate their NAC in almost any common cell chassis from a wide array of domestic cell lines including yeast, bacteria, plants, immortalized mammalian cell lines such as HEK293 or CHO cells, or induced pluripotent stem cells (iPSCs). However, the development of the NAC in embryonic stem cells (ESCs) is explicitly prohibited, and proposals including plans to develop for or test in ESCs will be deemed out of scope for the GO program. Regardless of the cell line choice, sequences made by performers will be restricted to proof of concept or for commercial applications only. No genes that would be export controlled or restricted for biosafety reasons will be within scope.

While it is expected that some construction of laboratory equipment may be required to test NAC designs, tasks and costs associated with these activities must be rigorously justified. Furthermore, the focus of GO is on integrating protein domains with known mechanisms for responsiveness to optical stimuli and synthesis of nucleic acids, and neither of these functions require substantial hardware development. In particular, control of optogenetic domains can be achieved with existing state-of-practice optical systems, and existing high-throughput sequencing capabilities are established even for single-cell and spatial assays. To this end, development of novel optical systems or sequencing platforms is unnecessary for success on GO, and it is therefore deemed out of scope for this program.

Program Structure:

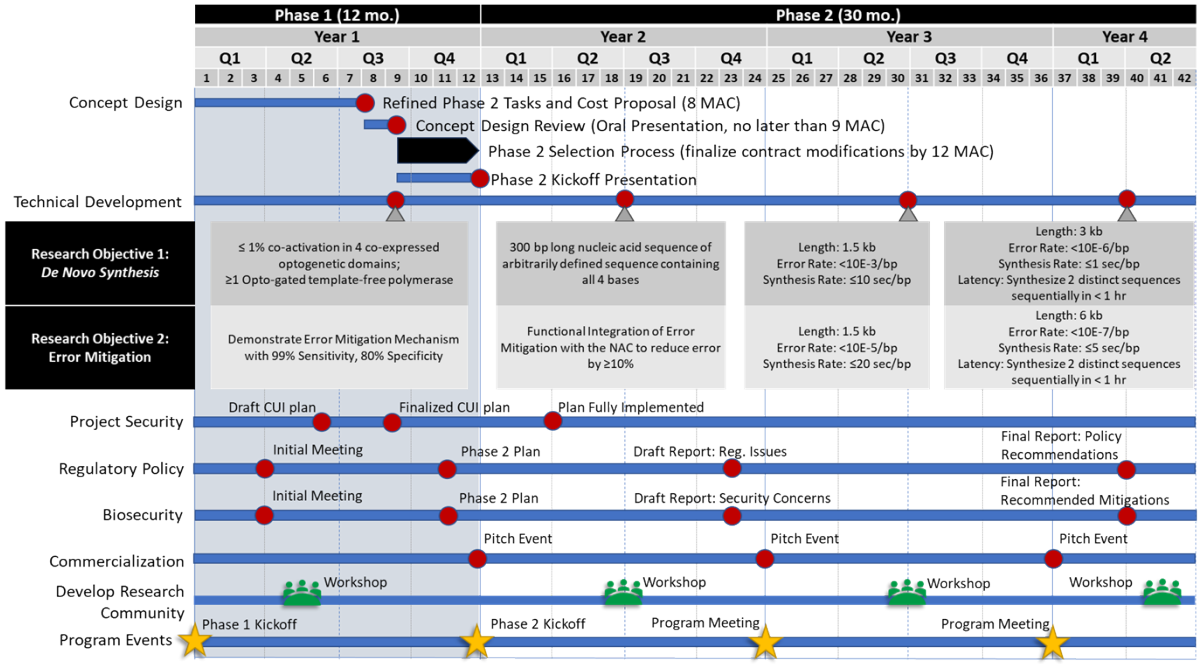

Figure 4. DRAFT GO program structure

The GO Program will proceed across two phases spanning a total period of performance of 42 months. Phase 1 (12 months) focuses on refining molecular components and de-risking integration strategies, while Phase 2 (30 months) focuses on integration and demonstration of the NAC platform *in vivo*.

Phase 1 (12 months): Performers will refine and demonstrate critical molecular components required for a NAC such as optogenetic domains, enzymatic polymerization, and error mitigation mechanisms. This phase provides teams with opportunity to de-risk their technical approach before beginning the more complex integration work required in Phase 2. Phase 1 also affords teams some time to address any capability gaps they identify through their technical progress. Specifically, Phase 1 is purposefully structured to allow teams time to reorganize themselves based on changes and refinements to their Phase 2 approach. For instance, informed by experimental results in Phase 1, a team may determine that they need to incorporate an additional or different expertise for success in Phase 2. This expertise could be obtained by bringing on a Co-PI or sub-awardee for Phase 2, and this individual or organization could be another party participating in Phase 1 or from outside the program.

To support the development of a research community around GO technology and to facilitate team refinement during Phase 1, the program incorporates a DARPA-sponsored workshop scheduled for month 5. All teams will be expected to present their research at this workshop. The workshop will be open to the members of relevant research communities beyond the performers on GO, following a registration and a review process for submitted workshop research synopses. In addition to providing GO performers with a venue to identify necessary

talent to augment their teams, this will be the seminal event to prompt conversations about GO technology, including barriers to broader adoption and norms that should be inculcated to foster responsible and ethical development of the technology. As a DARPA-sponsored event, the Agency will make all decisions regarding attendees. However, to inform whether non-performer attendees at this event are planning to brief capability of interest to GO performers, all Phase 1 performers on GO will be expected to provide DARPA statements of interest/disinterest in research synopses submitted by de-identified non-performers who wish to attend the workshop.

By month 9, performers must present their refined Phase 2 plan during a Concept Design Review (CoDR), including any planned restructuring of their team. The CoDR serves as a critical down-selection point, requiring performers to present compelling technical plans supported by demonstrable progress in Phase 1 to de-risk those plans. At the CoDR, performers will also need to communicate a project plan, including any changes in teaming, to establish confidence that the technical work is achievable within the required timeframe for the program. Key technical and project risks ensuring that only the most promising approaches are selected to continue and contribute to the program's success. The final three months of Phase 1 will focus on initial integration of technologies into a prototype NAC and preparation for the first commercialization pitch event, which will coincide with the Phase 2 kickoff meeting. While it is anticipated that only a subset of Phase 1 teams will move on to Phase 2, oral presentations in response to this solicitation should include plans to use the last three months of Phase 1 in preparation for Phase 2.

Phase 2 (30 months): Phase 2 focuses on integration of the components developed in Phase 1 to create a functional NAC and ultimately demonstrate functionality *in vivo.* The first major technical milestone in Phase 2 (month 19) requires performers to demonstrate their NAC design either cell-free (*in vitro*) or *in vivo*. However, by the second milestone (month 31) all demonstrations for both RO1 and RO2 must be completed in a living cell, showcasing the full functionality of the NAC platform.

Program Metrics:

The GO program contains four major technical milestones spread across the two program phases, and each of these milestones is associated with a set of quantitative metrics (Table 1) designed to assess whether GO program performers are making sufficient technical progress to justify their continuation on the program. Ultimate success on the program involves meeting or exceeding the final metrics in Phase 2 via NAC design that functions in a living cell (i.e., *in vivo*) to synthesize nucleic acids template-free. Of note, the metrics listed in Table 1 represent the minimal set of metrics used to define success on the program, and these DARPA-defined metrics are system-level requirements that are generally agnostic to performer-specific approaches. Proposing teams are strongly encouraged to include additional quantitative metrics to characterize success reflective of specific aspects of their unique NAC design. Similarly, DARPA reserves the right to include additional metrics to awards made under GO on a per team basis as necessary to manage risks unique to individual teams' different technical

strategies for designing a NAC.

In Phase 1, metrics associated with the month 9 milestone correspond to the timing of the CoDR. Teams are expected to meet these metrics in the process of de-risking the Phase 2 integration of the NAC. For RO1, the metrics aim to identify and develop at least four optogenetic domains responsive to different light wavelengths, enabling precise incorporation of the four nucleotide bases into a sequence. Performers must also demonstrate that these optogenetic domains can be integrated into a polymerase.

RO2 performers must meet the RO1 metrics and demonstrate a mechanism for error detection that is highly sensitive. Since multiple NACs will eventually be expressed in a single cell at the same time, less specific mechanisms will occasionally mislabel correct sequences as those containing an error. The metrics for RO2 are established under the assumption that over assessing errors will limit yield of NAC-produced nucleic acids per cell, if error containing sequences are filtered out of the population. However, any reduction in yield could be overcome by increasing the expression level of NAC proteins in the cellular chassis. Thus, RO2 metrics at this milestone are established to bias development toward highly sensitive approaches to error mitigation. To simplify experimental preparation and ensure a high degree of control over experiments needed to demonstrate NAC components, both RO1 and RO2 teams must generate functional datasets to meet metrics for the month 9 milestone from *in vitro* (i.e., cell-free) preparations. Optionally, proposers may also include work *in vivo* as part of their strategies to de-risk Phase 2.

For teams that advance into Phase 2, the RO1 metrics associated with the month 19 milestone are structured to demonstrate an early version of a NAC capable of integrating four optogenetic domains to regulate incorporation of each nucleotide species into a 300-mer oligonucleotide. This milestone does not include an error rate for RO1. However, RO2 performers must demonstrate a 10% relative reduction in error as part of their early integrated NAC design. RO2 performers should include experimental plans with necessary control conditions and/or NAC

Table 1. DRAFT GO program metrics by milestone and RO

	Research Objectives	
Program Milestones:	**RO1: De Novo Synthesis**	**RO 1 + 2: Error Mitigation**
Phase 1: Month 9 Advancement Criteria	In vitro demonstrate: • ≤ 1% co-activation in 4 co-expressed optogenetic domains • ≥1 Opto-gated template-free polymerase	In vitro demonstrate: • ≤ 1% co-activation in 4 co-expressed optogenetic domains • ≥1 Opto-gated template-free polymerase In vitro demonstrate: error mitigation mechanism with 99% sensitivity, 80% specificity
Phase 2: Month 19 Milestone	In vitro or in vivo demonstrate: • 300 bp long nucleic acid sequence of arbitrarily defined sequence containing all 4 bases	In vitro or in vivo demonstrate: • 300 bp long nucleic acid sequence of arbitrarily defined sequence containing all 4 bases In vitro: functional integration of error mitigation with the NAC to reduce error by ≥10%
Phase 2: Month 31 Milestone	In vivo demonstrate: • Length: 1.5 kb • Error Rate: <10E-3/bp • Synthesis Rate: ≤10 sec/bp	In vivo demonstrate: • Length: 1.5 kb • Error Rate: <10E-5/bp • Synthesis Rate: ≤20 sec/bp
Phase 2: Final Metric	In vivo demonstrate: • Length: 3 kb • Error Rate: <10E-6/bp • Synthesis Rate: ≤1 sec/bp • Latency: Synthesize 2 distinct sequences sequentially with < 1 hr between the 1st and 2nd sequence	In vivo demonstrate: • Length: 6 kb • Error Rate: <10E-7/bp • Synthesis Rate: ≤5 sec/bp • Latency: Synthesize 2 distinct sequences sequentially with < 1 hr between the 1st and 2nd sequence

designs that lack a functional error mitigation mechanism in their proposals. RO1 performers must show optically controlled incorporation of each nucleotide species, avoiding random incorporation merely triggered by illumination. RO1 experiments may be conducted *in vitro* or *in vivo*, RO2 metrics should include *in vitro* data as a minimum, with optional *in vivo* results for additional validation.

At a general level, GO performers will need to implement experimental approaches to demonstrate optically controlled incorporation of specific nucleotides and characterize error rates of their NAC design. OPPs in response to this solicitation must describe experimental designs and analytical approaches to quantify NAC-produced sequences from both *in vitro* and *in vivo* experiments and how these approaches will be used to address metrics at each milestone. GO performers may pursue approaches to demonstrate the spatial resolution of their overall system (e.g., optically writing different nucleic acid sequences in adjacent cells), and the abstracts and OPPs should be clear that a team intends to pursue these experimental demonstrations during Phase 2. In this case, the OPP should include a description of the sequencing approaches necessary to demonstrate this experimentally.

Experimental approaches to test and demonstrate NAC functionality will need to include strategies for refining the parameterization of optical signals used to encode the nucleic acid sequence. Teams should include a brief discussion of their notional strategy to design and experimentally refine these optical signal characteristics in their OPP, and this should be clearly delineated from refinements to the design of the NAC itself or the cellular host expressing the NAC. This section of the OPP should be clear how combinatorics of the elements of overall system (NAC design, different cellular chassis, and optical signal design) can be minimized to facilitate tractable development plans to meet progressively more difficult metrics over the timeline of Phase 2.

The months 31 and 41 milestones correspond, respectively, to the penultimate and ultimate sets of metrics that Phase 2 GO performers must meet. Unlike previous milestones, the metrics for RO2 performers supersede the RO1 metrics. Specifically, because RO2 performers will include additional error mitigation mechanisms into their NAC designs, it is assumed that error detection and the mitigation down-stream of the detection will require some time. Thus, the metric for synthesis rate is relaxed relative RO1, but the ability to synthesize sequences with significantly reduced error rates should facilitate the synthesis of longer, usable sequences compared to RO1. While supporting results may include *in vitro* experiments, key data for both RO1 and RO2 performers demonstrating successful completion of month 31 and 41 milestones must come exclusively from *in vivo* experiments.

A key attribute of GO systems is the ability to use optics for massless information transfer to living cells, and accordingly, successful technologies developed on GO should facilitate short-latency sequential transmission of multiple genetic sequences to the same cell. Accordingly, as part of the month 41 milestone all GO performers must demonstrate the ability to write two different nucleic acid sequences to the same cell or population of cells with less than 1 Hr to reset the GO system between write events.

Table 2. DRAFT Test matrix for Phase 2 milestones at months 31 and 41. DARPA will provision performer teams with a common set of sequences aligned to the table above. For coding sequences provided by DARPA, performers may work with DARPA to adjust these sequences (e.g., codon optimization), so it is possible to make functional assessments of gene products' effects within the context of the performer's cellular chassis. Sequence adjustment will be allowable provided it is possible to maintain the desired GC content and number of recombination sites. If this is not possible and the performer has evidence of successful NAC transduction of the optical signal encoding the sequence, then it will be the performer's responsibility to provide an alternative test sequence aligned to the GC content and N recombination sites of the problematic sequence. This sequence will be in addition to the minimum number performers are required to provide. As part of their OPP, proposing teams are strongly encouraged to discuss how they will populate the 49 sequences they are responsible for in the table above.

Sequence Complexity	Sequence Function	*N* Sequences		Overall %GC Content	*N* Recombination Sites
		DARPA-Provided	*Performer-Provided*		
Low	Coding	2	≥2	50+/-5%	0
	Non-coding	3	≥5	50+/-5%	0
Medium	Coding	2	≥2	35+/-5%	0
		2	≥2	75+/-5%	0
		2	≥2	50+/-5%	2
	Non-coding	3	≥5	35+/-5%	0
		3	≥5	75+/-5%	0
		3	≥5	50+/-5%	2
High	Coding	2	≥2	35+/-5%	4
		2	≥2	<30%	0
		2	≥2	>80%	0
	Non-coding	3	≥5	35+/-5%	4
		3	≥5	<30%	0
		3	≥5	>80%	0
Total Sequences Tested:		**35**	**≥49**		
Grand Total:		**≥84 sequences**			

The metrics associated with error rate for the months 31 and 41 milestones represent absolute maximum error rates, but they do not prescribe sequence-specific error rates. Existing approaches for *de novo* nucleic acid synthesis do not exhibit sequence-independent and uniformly distributed errors, and some sequences, like those with high GC content, are particularly problematic due to their propensity to form secondary structures that inhibit current template-free synthesis mechanisms. It is not clear that GO technologies will be susceptible to the same conditions that determine sequence-specific error rates for other technologies, but it would be imprudent to assume that GO technologies will be immune to sequence-specific error probabilities. Therefore, while GO performers will not be required to meet metrics assessing the sequence specificity of error rates, they will be required to characterize what those rates are

within the bounds of sequences that are known to be problematic to existing *de novo* DNA/RNA synthesis technology. As such, DARPA will provision GO performers with a common set of test sequences as described in Table 2, but proposals should plan to discuss how they will augment these sequences with additional sequences relevant to their intended cellular chassis and NAC design. It is expected that these plans will be revised during Phase 1 and the finalized plans will be described in the CoDR at month 9.