Program Solicitation

for

Simulated Microbial Systems (SMS)

Biological Technologies Office (BTO)

DARPA-PS-25-04

November 26, 2024

PROGRAM SOLICITATION OVERVIEW INFORMATION

- **Federal Agency Name** – Defense Advanced Research Projects Agency (DARPA), Biological Technologies Office (BTO)
- **Funding Opportunity Title** – Simulating Microbial Systems (SMS)
- **Announcement Type** – Initial Announcement technical
- **Funding Opportunity Number** – DARPA-PS-25-04
- **Dates**
  - Industry Day: August 26-27, 2024
  - Posting Date: November 26, 2024
  - Questions Due Date: December 13, 2024
  - Abstracts Due Date & Time: January 15, 2025, 1200 Eastern
  - Oral Proposal Package (OPP) Due Date & Time: February 25, 2025, 1200 Eastern
  - Oral Presentation Date: March 5, 2025
- **Description of the funding Opportunity:** The Defense Advanced Research Projects Agency (DARPA) is soliciting proposals to create generalizable and comprehensive computational simulations to predict the behavior of individual *Escherichia coli*. Simulation software will also be tailored to forecast bacteria function in various user-defined scenarios. This program also seeks innovative experimental workflows to measure *E. coli* to generate data to parameterize and validate simulation software. Innovations to simulate and measure *E. coli* will inform one another and accelerate progress towards the ultimate goal – a generalizable and comprehensive simulation software platform to predict *E. coli* behavior.
- **Multiple awards are anticipated**.
- **Total Funding** – DARPA has approximately $15.8M total for performer awards and anticipates making multiple awards.
- **Types of instruments that may be awarded** – Other Transaction for Prototype
- **Technical Point of Contact** – Dr. Chris Bettinger, Program Manager
- **Agency Contact**

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- **Attachments**
  - A. Abstract Summary Slide Template
  - B. Abstract Template
  - C. Model Other Transaction (OT) for Prototype, Fixed Support
  - D. Cost Spreadsheet
  - E. Schedule of Milestones and Payments
  - F: Controlled Unclassified Information (CUI) Guide

PROGRAM SOLICITATION
Defense Advanced Research Projects Agency (DARPA)

1. PROGRAM INFORMATION

1.1. Background

Nobel laureate Francis Crick outlined his vision for fully resolving the K12 strain of *Escherichia coli* in a paper he published in 1973 entitled "Project K: The Complete Solution of *E. coli*". This radical vision outlined a path to reconcile molecular biology, biochemistry, and systems biology. It also sparked the imagination of scientists and catalyzed countless inventions and discoveries in the following decades. The last 50+ years have seen remarkable advances in experimental tools to interrogate biological systems at the molecular level, in software to simulate them, and in high-performance computational and storage platforms to handle large datasets. In short, the goal of the Simulating Microbial Systems (SMS) program is to work towards realizing Francis Crick's vision of fully resolving the K12 strain of *E. coli* by combining high-throughput molecular biology with advanced software to create generalizable and extensible multi-scale computational simulations to predict the behavior of *E. coli*.

Recent advances in computational biology can predict various biological components including protein structure, gene transcription/transcriptional networks, and metabolic pathways. Furthermore, various subsystems have been combined to create "whole-cell models" (WCMs) that can predict aspects of the intracellular behavior of simplified synthetic cells, which contain ~400 genes. However, to date, WCMs and related approaches cannot predict the behavior of more complex bacteria (e.g., *E. coli*). Bacteria such as *E. coli* contain orders of magnitude more genes and more complicated interactions (see **Figure 1**).

There are many practical and technical challenges to simulating an *E. coli* bacterium. These challenges are best contextualized when compared with simulations of minimal synthetic cells:

1. Gaps in Data: Approximately 1500 (35%) of *E. coli* proteins have uncertain function. The total amount of proteins with uncertain function is 30x larger in *E. coli* compared to in a minimal cell (~40 proteins, 10%).
2. Increased Size & Complexity: Bacteria are orders of magnitude more complex than minimal cells. *E. coli* have 10x more genes and are 15x larger in volume.
3. Simulating Stochastic Processes Across Length Scales: Biological processes are inherently stochastic. Stochastic processes at the molecular scale (e.g., ligand-receptor binding, diffusion) produce large variations in higher-order observables (e.g., doubling time, DNA replication).

Computational simulations of *E. coli* will radically transform molecular biology and biotechnology by allowing wet lab experiments to be performed *in silico*. For example, simulation software could be leveraged as an engineering design tool to accelerate design-build-test-learn (DBTL) cycles in synthetic biology and biomanufacturing. A faithful and reliable simulation of bacterial behavior may also be used to predict the efficacy of novel medical countermeasures.

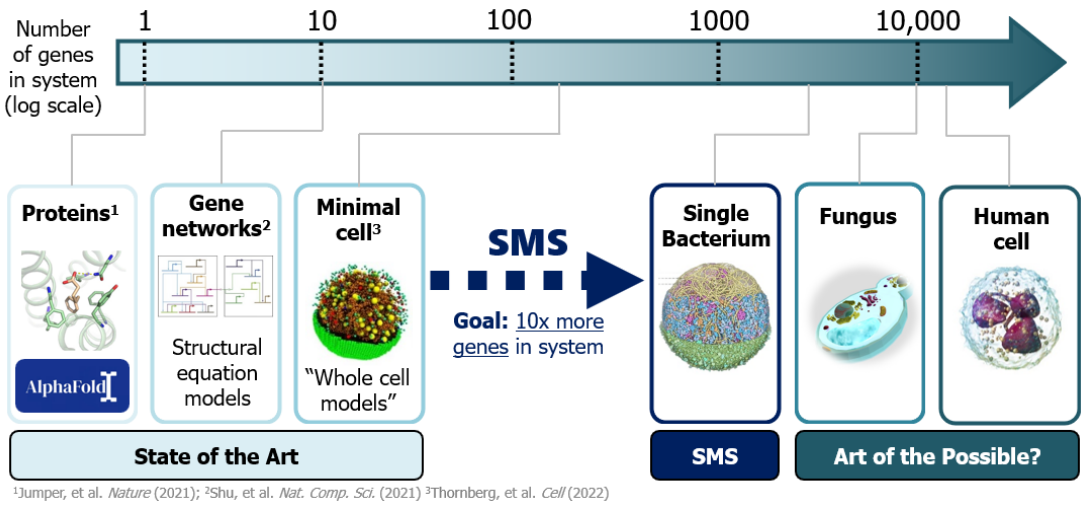

Figure 1 *SMS aims to revolutionize the state of the art in biological system simulation.*

1.2. Program Description/Scope

The Simulating Microbial Systems (SMS) program is an 18-month effort that seeks to create comprehensive, generalizable, and extensible computational simulations to predict the properties and behavior of a single *E. coli* K12 bacterium. The program will unify recent advances in biological modeling, molecular biology, and systems biology to create software to simulate *E. coli* behavior by simulating processes across multiple relevant length- and time-scales (e.g., diffusion-reaction of biomolecules, transport, receptor-ligand binding, metabolic networks, gene transcription/regulation, and protein translation). Various sub-cellular processes would ideally inform inferences about and predictions of the temporal evolution of higher-order processes and properties (e.g., cell size, doubling time). Extensible simulations will also be directed at two use cases that have Department of Defense (DoD) relevance: accelerating design-build-test-learn (DBTL) cycles in biomanufacturing; predicting antimicrobial efficacy.

Performer teams will interact with one another and the US Government (USG) team to evaluate, improve, review, and ideally transition and release research (see **Figure 2** and **Figure 3**). Performers will be concurrently generating data and creating simulations. The USG team will consist of DARPA and Independent Verification and Validation (IV&V) partners. The USG team will generate ground truth data and work with performers to assess progress and ensure operability of simulation software. Performer progress will be assessed by capability demonstrations (CDs) and pressure tests (PTs). Two CDs (and corresponding workshops) will assess the operability and extensibility of simulations. PTs will assess performance in unique operational constraints described by DARPA. During CD and PT, performers will help IV&V run simulations on USG systems for evaluation. The USG team will work with performers to improve software and help facilitate transition to potential partners.

In accordance with the Controlled Unclassified Information (CUI) guidance for SMS, all simulation code, results, and data produced in the program will initially be controlled at the CUI level. These controls also aid evaluation and transition by increasing operability with USG systems. DARPA anticipates that SMS will produce unclassified, fundamental research. Subject to a review by DARPA, unclassified information can move off CUI-compliant information systems. Section *1.11.1* provides additional details on SMS's CUI controls, and Section *1.11.2* describes DARPA-facilitated access to CUI systems for optional performer use (e.g., AWS GovCloud). DARPA requires a second pre-publication review of specific materials to establish public releasability (see Section *1.12*). Workshops will afford an opportunity for review, and will include performers,

DARPA, IV&V, and the program's Ethical, Legal, Societal Implications (ELSI) group. SMS will ultimately produce software that will be furnished to stakeholders including the DoD while experimental data is anticipated to be published to online public repositories.

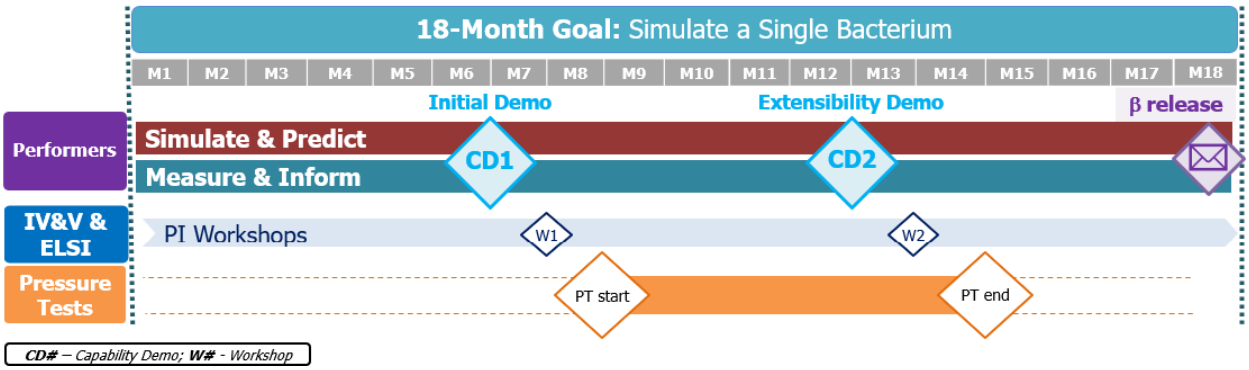

Figure 2 *SMS schedule (anticipated). Performers will participate in two CDs and two PTs. CDs will assess simulation operability and extensibility while PTs will assess performance in unique operational constraints. Performers, the USG team, and ELSI group will participate in workshops after each CD for discussion of results. Workshops will also allow review of simulations by DARPA and IV&V partners. Beta releases will be furnished to DoD stakeholders.*

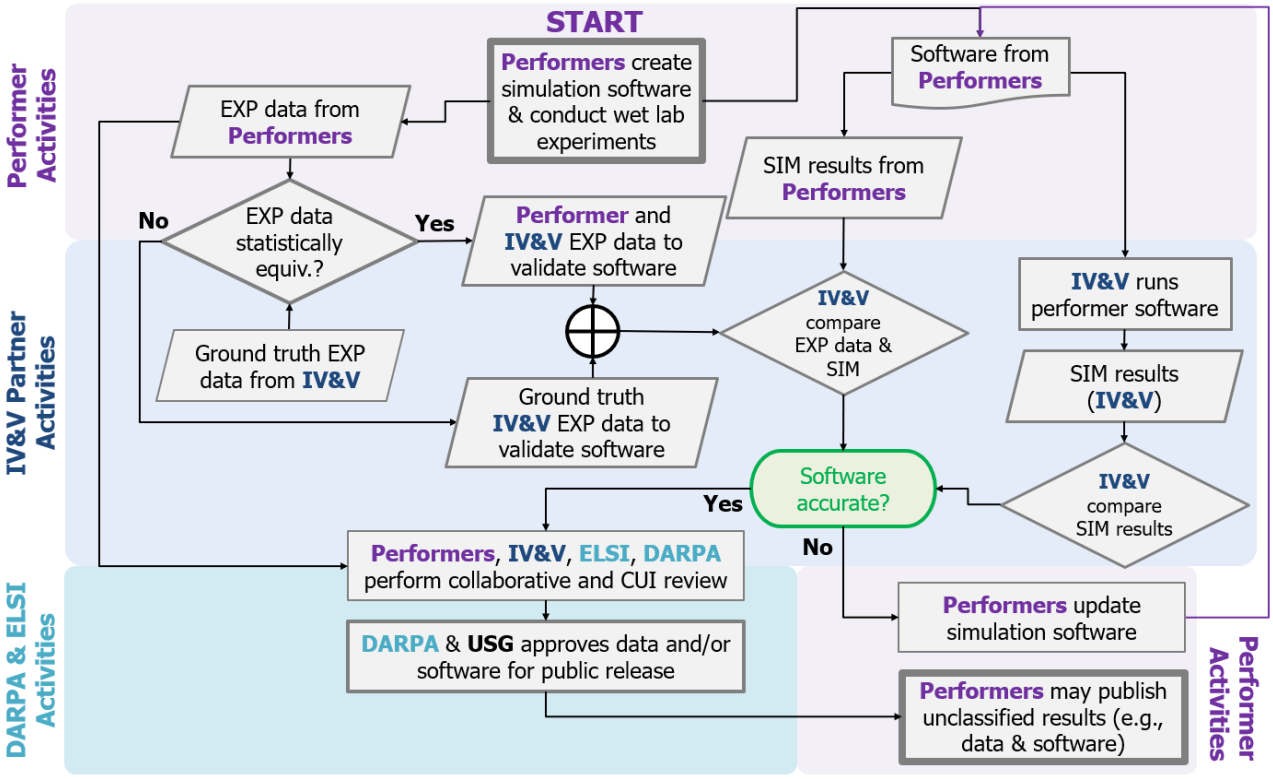

Figure 3 *Generic workflow between SMS performers, IV&V partners, ELSI group, and the DARPA team. Performers will concurrently produce simulations and data, and will help IV&V run software on USG systems for evaluation. After review, performers may publish any unclassified results.*

1.3. Acquisition Strategy

SMS is using a modified acquisition approach to lower the administrative burden of entry,

reduce program risk, foster competition, and accelerate start dates for performer teams. This Program Solicitation (PS) solicits independent abstract submissions for an 18-month effort. Successful abstract proposers will be invited to provide an oral presentation to present their proposals to the DARPA SMS program team. The Government will review all oral presentations, and selected proposers may be awarded an Other Transaction (OT) for Prototype agreement. This PS 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, universities, and research institutions. SMS will initially focus on simulating individual *E. coli*. While future efforts to simulate multiple cells and types may be considered in the future, follow-on expansions are NOT solicited in this PS. See *1.11.3* for more information.

1.4. Program Structure

1.4.1. Unified Research Thrusts

The SMS program will be composed of two complementary research thrusts: (1) Measure & Inform; and (2) Simulate & Predict. ***Proposals must describe credible plans to conduct research in both research thrusts simultaneously***.

1. Measure & Inform. Though widely studied, the K12 strain of *E. coli* has significant gaps in data. Even amongst the presently available data (e.g., EcoCyc, GenoBase, KBase), much of the information lacks proper experimental context and/or standardized metadata. The Measure & Inform thrust will generate large datasets that describe the dynamics of *E. coli* K12 properties with the goals of filling in these gaps in data and informing simulation development, parameterization, training, and testing. These data will be published to public repositories in accordance with applicable law, regulations, and policies. Successful approaches to complete the Measure & Inform thrust are anticipated to use emerging technologies that include, but are not limited to:

- Automation, robotics, computer vision, and other platforms to enable high-throughput "human out-of-the-loop" experiments.
- Microfluidics, microsystems, and precision nanoliter-scale handling of fluids.
- Imaging, microscopy, spectroscopy, and associated downstream data processing.
- Single-cell analytical techniques including multi-omics (e.g., genomic, proteomics, metabolomics and lipidomics).

Proposals should describe how they will acquire data relevant to broadly applicable properties and behaviors of an *E. coli* bacterium, and data related to tailoring to specific DoD use cases (see *1.4.2* and *1.5*). Proposals should describe how their approaches: will properly parameterize and inform simulations; create relevant data including relevant metadata. Proposals that do not plan to generate significant datasets but instead leverage only pre-existing datasets will be considered non-responsive. The Government team will furnish an *E. coli* K12 to performers which will serve as the standard for the SMS program. Alternatively, performers may describe their own *E. coli* K12 strain with justification(s). All alternative strain(s) must be shared with the program. DARPA reserves the right to review and approve alternatives. If a proposed alternative strain is not approved, the program will provide a strain at kickoff. Final strains are to be determined at time of award negotiation and are subject to DARPA approval.

2. Simulate & Predict. The goal of the Simulate & Predict thrust is to create generalizable, extensible, and interoperable software that can forecast or predict the properties and behavior of an *E. coli* K12 bacterium. Proposals should describe approaches to simulate stochastic biological processes across many length scales (see Section *1.1*) and should describe how their choice of simulation techniques may impact Measure & Inform. Proposals should

describe how software will be parameterized using: newly generated data from the Measure & Inform research thrust; data from publicly available databases. Proposals should include hardware or cloud estimates of storage, compute, and any other services (e.g., batch compute, datastores, on-demand parallel clusters for HPC, GPUs, software development / ML sandboxes, etc.). Lastly, proposals should describe plans for interoperability and compatibility with the computing platforms of Government partners and stakeholders that handle Controlled Unclassified Information (CUI; see *1.11.1*; and see *1.11.2* for DARPA-facilitated CUI options). Successful approaches to complete the Simulate & Predict thrust are anticipated to include, but are not limited to:

- Computational biology, physics-based modeling, and multi-scale modeling.
- Artificial Intelligence & Machine Learning including, but not limited to, computer vision, large language models, constitutive law models, neuro-symbolic programming, tensor decomposition methods, synthetic data generation.
- Probabilistic and statistical approaches, including, but not limited to, inverse probability models, Bayesian workflows, causal inference, time series forecasting, uncertainty quantification.

Technical abstracts and oral presentations should present innovative approaches in each research thrust and a cohesive workflow that integrates them (see, notionally, **Figure 4**). ***Proposers are expected to be comprised of dynamic, interdisciplinary, and potentially multi-institutional teams with expertise that collectively spans both research thrusts***. Proposals that prominently feature incremental improvements of approaches will be considered non-responsive.

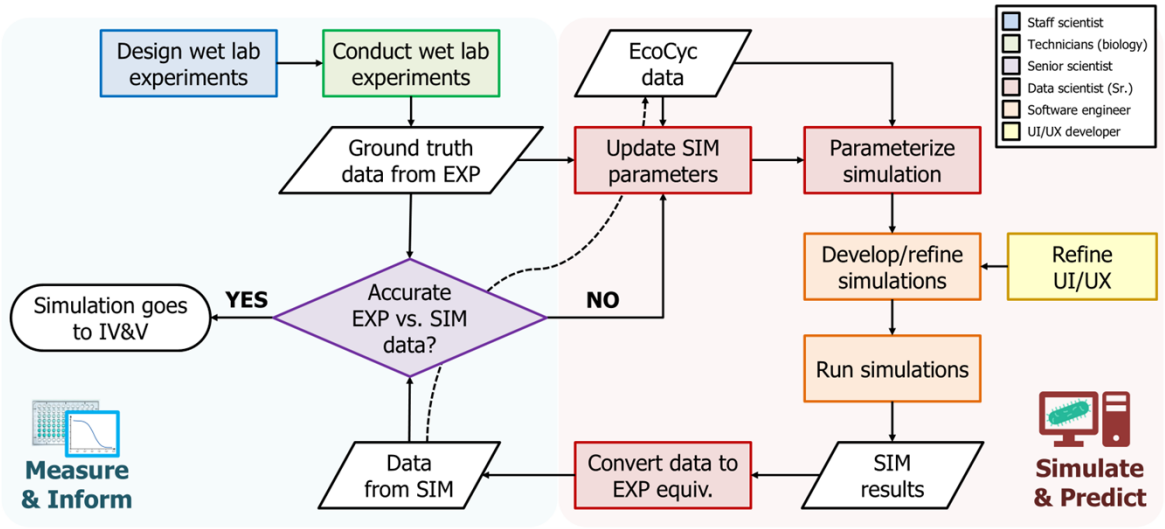

Figure 4 *Notional teaming and workflow for performers to integrate research thrusts of SMS.*

1.4.2. SMS Scope: Simulation Generalizability & Compatibility with Specific Use Cases

Generalizability. Each proposal should plan to create a generalizable software platform that can be tailored to simulate *E. coli* in different potential use cases that would be of interest to the DoD. To this end, simulations should track the spatio-temporal dynamics of important biomolecules that comprise an *E. coli* (e.g., abundance of specific mRNA, proteins, metabolites, and lipids) and should also predict higher-order behavior (e.g., growth kinetics); see metrics in Section *1.6.1*. Proposals should include information about how to establish simulations' equivalence with ground truth data gathered from experiments (see *1.6.2*).

Specific Use Cases. SMS will assess this capability in the context of two use cases: (1) Design a Biomanufacturing Chassis; (2) Predict Antimicrobial Efficacy.

(1) *Design a Biomanufacturing Chassis*. Performers will use simulations to design and optimize an *E. coli* K12 chassis that can produce high-value small molecules of interest to the DoD. SMS suggests limonene or violacein, although proposers may suggest alternative compounds with appropriate justification. DARPA reserves the right to review and approve alternatives. Final assignments are to be determined at time of award negotiation. NOTE: Protein-based biomanufacturing products are out of scope for SMS. The DARPA SMS team recognizes the *E. coli* K12 strain is not an optimal chassis for biomanufacturing. The goal is not to maximize overall production relative to other chassis organisms. Rather, performers should demonstrate how software simulations can be used as an engineering design tool to optimize strains and accelerate DBTL cycles in biomanufacturing.

(2) *Predict Antimicrobial Efficacy*. Performers will use simulations to predict the minimum inhibitory concentration (MIC) of an existing antibiotic on *E. coli* K12. Performers will be assigned at least one antimicrobial from a list of pre-existing antimicrobial compounds that will be drawn from one of the following classes: β-lactams, fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole. Proposers should indicate any preferences for assignments with appropriate justifications. DARPA reserves the right to review and approve preferences. Final assignments are to be determined at time of award negotiation.

1.5. Program Events and Evaluation Points

1.5.1. Capability Demonstrations (CDs)

Performer progress will be measured by successful completion of two CDs. CDs are two structured opportunities for performers to showcase progress in data collection and simulation. Performers will demonstrate an initial version of the simulation software in CD1. Performers will extend simulation software capabilities in CD2. Performers may work towards completing both CD1 and CD2 concurrently and immediately. It is anticipated that CD1 and CD2 will conclude on Months 6 and 12, respectively. Performers will showcase technical progress during in-person workshops (see **Table 6**). Detailed descriptions of CDs are shown in **Table 1**, and associated metrics are shown in **Table 2**, **Table 3**, **Table 4**, and **Table 5**. Proposers should describe how their activities and approaches will enable them to complete CD1 and CD2.

1.5.2. Specific Requirements of Capability Demonstrations

Table 1 *Detailed description of Capability Demonstrations (CDs).*

Capability Demonstration (Anticipated Due Date)	**Overall Goals and Specific Requirements of Capability Demonstration**
CD1 – Initial Demonstration (6 months)	Performers will demonstrate initial versions of simulations to predict biomanufacturing yield and antimicrobial efficacy. Simulations will also predict overall cellular composition, properties, and higher-order behavior. To complete CD1, the performer will: • Deliver source code and simulation software to both Government team(s) and Independent Validation and Verification (IV&V) partners. • Provide the US Government and IV&V partners with appropriate background, training, orientation, and technical support. • Run simulation code/software on IV&V systems.

	• Present technical results to the DARPA SMS team, IV&V partners, and associated stakeholders (1-hour presentation followed by Q&A). • Receive and incorporate IV&V experimental "ground truth" data and accompanying equivalence analysis results (see *1.6.2*). • Participate in a DARPA-sponsored workshop to demonstrate software capabilities and features (see *1.5.5*). • Meet metrics relevant to CD1 (see **Table 4**) • Demonstrate progress towards and a credible plan to meet additional metrics (see **Table 2**, **Table 3**, and **Table 5**).
CD2 – Extensibility Demonstration (12 months)	CD2 will expand upon CD1 by including additional perturbations to assess simulation extensibility. To complete CD2, the performer will: • Repeat all tasks previously listed in CD1 (see above), including meeting metrics related to use cases (see **Table 4**), plus further progress towards achieving metrics for forecasting molecular concentrations and higher-order properties (see **Table 2**, **Table 3**, and **Table 5**). • Simulate *E. coli* behavior in response to perturbation(s) (see below). Pressure tests (see *1.5.3*) will incorporate relevant subsets of perturbations, and CD2 may further test others. Details will be provided after CD1. • Genetic Modification: Performers may predict the response of *E. coli* to a genetic modification (e.g., a new plasmid, gene deletion or modification, phage infection, UV stress, horizontal gene transfer). • Chemical Perturbation: Performers may predict *E. coli* behavior in response to changes in the chemical environment (e.g., presence of an unusual small molecule, absence of a carbon source, different pH values, alternative carbon/nitrogen sources, different redox potentials).

1.5.3. Pressure Tests (PTs)

SMS performers will complete two pressure tests (PTs). PTs will demonstrate that simulations can adapt to unique operational constraints provided by DARPA. Proposers should describe how they will meet the goals and metrics of each PT. Proposals that plan for PT models that are entirely disconnected from and independent of their simulations used for CDs will be considered non-responsive. PTs will be performed concurrently with CDs. It is anticipated that PTs will start on Month 8 and conclude on Month 14. Performers will deliver simulations to IV&V partners at least once for each PT, helping them run on IV&V systems, and will present results to the USG team. It is anticipated that IV&V will perform final assessments on PTs by Month 15. The general scope of each PT is detailed below. NOTE: Details of each PT will be provided after CD1.

1.5.4. Specific Requirements of Pressure Tests

(1) *Pressure Test #1: Simulate Production of High-value Molecules at Different Operating Conditions.* Performers will use simulations to predict the production of their assigned compound in DARPA-selected physical environments that mimic the various differences in operating conditions that correspond to different production scales. This PT may manifest as the absence/presence of shear stress or variations in dissolved oxygen and temperature.

(2) *Pressure Test #2: Predict Efficacy of an Alternative Antimicrobial Compound*. Performers will use simulations to predict the MIC of a second antimicrobial (i.e., different from their initial assignment). The DARPA-selected antimicrobial will be drawn from the classes of compounds used for CDs. In addition, DARPA may specify perturbations, stressors, or environmental changes. Examples include previous exposure to antibiotic; change in

pH/temperature; or change in cell state/growth cycle when antibiotic applied/eliminated.

1.5.5. Workshops

SMS will convene two (2) in-person collaborative workshops between performers and the USG team including IV&V partners, DARPA, and ELSI group members. Workshops will be two days, will occur approximately one month after each CD, and may include DoD stakeholders. Workshops will permit collaborative review of documents, source code, raw data, results, and parameters. Workshops will serve as a forum to review and discuss best practices for responsible software development by considering ELSI (see Section *1.8*).

Workshops will also be an opportunity to engage with the DARPA team to discuss details that might otherwise fall outside the scope of a routine technical brief, progress towards milestones and scientific goals, and any ongoing technical or programmatic challenges that must be overcome to achieve the overarching goals of the program.

1.6. Program Metrics

1.6.1. Metrics Associated with Capability Demonstrations (CDs)

Progress towards completing each CD will be assessed by metrics related to each research thrust (**Table 2** and **Table 3)** and use case (**Table 4**). Performer progress in CDs will also be evaluated by tracking how many properties and behaviors performers can equivalently simulate (**Table 3, Table 5**). Proposals should describe credible plans to meet these metrics and simulate these properties and behaviors. Measure & Inform metrics in **Table 2** will assess the performers' ability to measure and interrogate the complex dynamic molecular composition of an individual *E. coli* bacterium.

Table 2 *E. coli properties to be measured experimentally, and CD metrics.*

Biological Property	Measure & Inform Metrics
• Gene transcription • Protein-protein interactions	• Measure concentrations of >4000 different mRNA molecules (single cell resolution; $>10^6$ measurements per time point) • Fully resolve and parameterize at least 2 different protein-interaction networks each comprised of >100 proteins
• Protein & metabolite concentrations (single cell)	• Measure concentrations of >400 proteins and >400 metabolites (single cell resolution; $>10^6$ measurements per time point)

Simulate & Predict metrics in **Table 3** will assess the simulation and prediction of experimental data gathered to meet Measure & Inform metrics in **Table 2**. The ultimate goal is to demonstrate statistical equivalence between *in silico* data from simulations (SIM) and *in vitro* data experiments (EXP) conducted by IV&V. Proposals should describe methods of establishing equivalence (see *1.6.2*).

Table 3 *Molecular properties of E. coli to be simulated, and CD metrics. Simulation results (SIM) will be evaluated against experimental data (EXP) generated by IV&V partners (see 1.6.2)*

Biological Property	Simulate & Predict Metrics

• Gene transcription • Protein-protein interactions	• Predict concentrations of all measured mRNA molecules vs. time (continuous) • Predict protein dynamics, regulation, and control within at least 2 different networks each comprised of >100 proteins
• Protein & metabolite concentrations (single cell)	• Predict concentrations of all measured relevant proteins and metabolites vs. time (continuous)

Success will be measured by assessing the degree by which simulated data from *in silico* simulations are statistically equivalent to experimental data generated through wet lab experiments conducted by IV&V partners. Individual comparisons will be conducted across thousands of proteins and small molecules. In addition to specific molecules, both *in vitro* and *in silico* data should aim to resolve protein interaction networks comprised of at least 100 proteins. Resolution includes a detailed interaction map and sufficient quantitative parameterization to make mechanistic predictions about protein dynamics, regulation, and control within the network (e.g., concentrations, reaction rates, equilibrium constants).

In addition to measuring and predicting sub-cellular dynamics at the molecular level, simulations should also demonstrate the applicable capability for each use case (**Table 4**). These evaluations will compare the data from simulations with data from IV&V *in vitro* experiments, using the latter as ground truth.

Table 4 *Description of CD1 and CD2 metrics associated with use cases. Both use cases should be evaluated during both CDs.*

Use Case	Technical Description and Metric
Biomanufacturing	• CD1 and CD2: Simulate the specific production rate to within ±10% of IV&V's experimentally determined baseline value. • CD2: Use software simulations to improve the design of the engineered *E. coli* K12 strain by increasing production by 10X over initial baselines.
Antimicrobial Efficacy	• CD1 and CD2: Simulate the MIC of a single assigned antimicrobial within ±10% of the experimentally determined value (as determined by IV&V). • CD2: Simulate the MIC of a cocktail of two antimicrobials within ±10% of IV&V's experimentally determined value.

Finally, multi-scale simulations should also predict relevant higher-order properties (**Table 5**). Simulations should be able to predict these properties by demonstrating statistical equivalence between results obtained from IV&V *in vitro* experiments and data generated by *in silico* simulations, using the IV&V data as ground truth. While notional methodologies to measure these parameters are shown below, the precise experimental methodology will ultimately be agreed upon between the performers and the prospective IV&V partners.

Table 5 *Higher-order properties of E. coli to be measured and simulated during CDs. These will be evaluated against IV&V experimental data; proposals should include metrics for that evaluation (see 1.6.2). Note: Parameters measured every 3 hours from 0 to 24 hours (minimum).*

Higher-Order Property	Parameter	Possible Experimental Techniques
Proliferation	Doubling time (min)	Optical density (log phase)
Cell mass	Cell mass (mg/10^9 cells)	Graviter method
Cell volume	Median cell volume (um^3)	Light scattering; microscopy

Genetic material	Total RNA & DNA content (# nucloeotides/g dry weight)	Fluorimetry
Metabolism	Glycogen (mmol glycosyl units/g dry weight)	Gas chromatography-mass spectrometry (GM-MS); Optical spectroscopy

SMS seeks unified simulations that can address these properties and behaviors; proposals that plan for individual, independent, or otherwise unlinked models for predicting each property or behavior will be considered non-responsive. Proposals should include simulation and measurement timescales, including how often and for how long each attribute, property, or behavior will be measured and simulated (e.g., every 1 sec, 5 min, 3 h, for 24 h, for 48 h, etc.). At the minimum, data generated from *in silico* simulations should have time steps that align with data generated from *in vitro* experiments (e.g., 0 to 24 h in 3h increments). The number of molecular properties and higher-order behaviors simulated (see *1.6.2*) will be a program metric.

1.6.2. Proposer-Specified CD Metrics to Establish Statistical Equivalence

SMS will evaluate simulations by testing statistical equivalence between "ground truth" from *in vitro* data obtained by experiments (hereby referred to as EXP) and *in silico* results obtained by running simulations (hereby referred to as SIM). Statistical equivalence between pairs of EXP and SIM datasets will be evaluated by IV&V for each property or behavior across the program (e.g., in **Table 3** and **Table 5**). Equivalence will be established when a given pair of EXP and SIM datasets are determined to be drawn from the same distribution (e.g., the null hypothesis is confirmed at $\alpha = .05$). Performers are encouraged to generate EXP and SIM data, and to compare them. For program evaluation, however, performers will assist IV&V in running simulations for SIM datasets, and EXP datasets generated by IV&V partners will serve as the "ground truth" and will thus be the ultimate arbiters to assess equivalence.

Proposers must specify equivalence metrics appropriate for their approaches. Suggestions include but are not limited to statistical tests such as Mann-Whitney U, Kolmogorov-Smirnov or chi-square; classification methods such as area under the ROC curve; forecasting methods such as Weighted Absolute Percentage Error, Mean Absolute Deviation, etc. Any approach and metric should be appropriately justified and should evaluate a hypothesis of equivalence at a significance level of 0.05 – i.e., only 5 times out of 100 will IV&V wrongly conclude that the simulation succeeded at matching the experiment. Final metrics are to be determined at time of award negotiation and are subject to DARPA approval.

1.6.3. Quantifying Stochastic Processes & Variance in Simulations

Proposers should clearly describe how their simulations can capture and leverage the underlying stochastic processes that are intrinsic to biological systems. Proposers should clearly describe how stochasticity at the molecular scale will map to anticipated variance observed in both molecular composition (**Table 2** and **Table 3**) and higher-order properties (**Table 5**). Furthermore, the simulation should be able to track the origin and quantity of the variance at all relevant length scales.

1.6.4. Optional Additional Metrics

Proposals may include additional metrics in the Measure & Inform or Simulate & Predict research thrusts to generate insight specific to their proposed approaches. Additional metrics should support the overall goal of the program and be justified accordingly. DARPA reserves the right to review and approve any additional metrics. Final metrics are to be determined at time of award negotiation and are subject to DARPA approval. Proposers should note that program metrics may

serve as the basis for determining whether satisfactory progress is being made.

1.6.5. Program Milestones

Suggested payable Milestones are listed in Attachment E. If selected, Proposers are encouraged to use these milestones as a baseline for creating their own. Milestones must be key observable events on the critical path to program execution. See Section *2.1* for more details.

1.7. Independent Validation & Verification (IV&V)

SMS performers will collaborate with IV&V partner(s). The primary functions of IV&V will be to: (1) Conduct wet lab experiments to generate "ground truth" EXP data; (2) run simulation software on USG systems.*1.7.1*

(1) Conduct Wet Lab Experiments. IV&V partner(s) will: independently conduct *in vitro* experiments and process their own data which will serve as the ground truth for the program; curate, standardize, and catalog the data and metadata from each performer during the program; compare results from each performer (including both *in vitro* and *in silico* results) with their ground truth data; perform analysis to evaluate simulation equivalence; ensure consistency in data creation across the program. The latter includes providing the same *E. coli* K12 strain to all performers who have not proposed and justified an alternative that has been approved by DARPA during contract negotiations, and receiving any alternative strains at program start.

(2) Run Simulation Software. IV&V partner(s) will: receive performer simulations for CDs and PTs, e.g., in a containerized manner; run simulations; validate simulation results. Simulations should run on simulation IV&V partner systems; performers should plan to work with IV&V partner(s) to facilitate this.

Collectively, IV&V teams will offer the performer the following capabilities and knowledge (at the minimum):

- The ability to properly handle and disseminate bacterial strains and necessary reagents.
- Expertise in cell and molecular biology to measure *E. coli* properties and behavior.
- Expertise in statistical and analytical techniques to support analysis of equivalency between *in vitro* data from experiments (EXP) and *in silico* results from simulations (SIM).
- The ability to run simulations, e.g., in a containerized fashion, after coordination with performers.
- The ability to interact with performers to improve simulations, e.g., by sharing ground truth data and metadata after CD and PT.

1.7.1. Proposer and IV&V Coordination

Proposers should include credible plans to have continuous and deliberate interactions with IV&V partners. Code and dataset delivery will be managed collaboratively by performers and IV&V partners. Proposals should include interim milestones justified by the technical approach that will address CD and PT (see Task Description Document [TDD] template & Schedule of Milestones and Payments). Proposals should describe notional plans to: handle program *E. coli* or share alternative *E. coli* strains; enable IV&V to run simulation code; utilize data and metadata from IV&V experiments and evaluations. Performer teams will be expected to establish an IV&V plan with IV&V partners as a deliverable early in the program (i.e., during CD1, see Task Description Document [TDD] template and Schedule of Milestones and Payments).

1.7.2. Simulation Progress Statistics

In addition, the program will collect the following simulation progress statistics:

- The number of time steps / attributes / properties / behaviors the simulation can predict in a 24-hour period.
- Relevant prediction and forecasting statistics (e.g., precision, accuracy, error)
- Algorithm runtime required, including e.g., training / retraining / fine-tuning time
- Number of genes/biomolecules/proteins/properties/etc. measured and simulated

Performers should plan to provide these statistics to DARPA at a minimum at each CD, at least twice during the PT period, and in the final report. Performers and IV&V may collaborate on collection and providing statistics.

1.8. Ethical, Legal, Societal Implications (ELSI)

SMS will include experts in ethics of emerging technologies, governance of artificial intelligence and machine learning, and community engagement. DARPA will engage experts to address potential ethical, legal, and societal implications of the performers' work. Independent ELSI group member(s) identified by DARPA will ensure that performer advances are safe and responsible. ELSI expert(s) will offer guidance on the ethics of simulations, predictions, comparison of biological data, and *in silico* outcomes. Proposers are encouraged to integrate experts into their teams to facilitate activities with DARPA and the external ELSI group. Proposals should include resources to engage ELSI group members at regular intervals to discuss responsible research and lessons learned, why decisions were made related to and how they were informed by ELSI. ELSI topics may include, and are not limited to:

- *What are the potential impacts of advancing the state of the art in cell modeling platforms?*
- *How can biologically faithful simulations inform scientific discovery and manage security?*
- *What details could genetic or chemical perturbations reveal or enable?*
- *Will prediction of MIC result in less potent antimicrobials or increasing resistance?*
- *How might open-source biomanufacturing design tools create vulnerabilities?*

1.9. Deliverables

All products, material, and otherwise that will be provided to the Government as outcomes from conducted research should be defined as part of the proposal. Performers should allocate resources for travel to workshops and transmission of deliverables. As applicable, the Government may include IV&V partners and other USG stakeholders.

1.9.1. Data Deliverables

All data will be delivered to the Government prior to each workshop, at the conclusion of each pressure test, and at the end of the program. These include modeling data and simulation results; experimental data; and relevant metadata. Any other datasets delivered to DARPA and IV&V partners may serve as a vehicle for informal feedback. Proposers should include relevant information in their proposal related to dataset format and delivery.

1.9.2. Simulation Deliverables

Simulations will be delivered to the USG team at minimum: at the end of CD1; at the end of CD2; and once for each PT. Simulation delivery includes executable code that runs on IV&V systems. Complete simulation software will also be delivered at the end of the program, including source code and any compiled executables; accompanying prerequisites or requirements of hardware, software and data; installation instructions, documentation, user manuals, and any other information to help the Government install and successfully execute simulations on its computer systems.

1.9.3. Other Deliverables

Other deliverables for SMS performers include:

- Data Management Plan (see Attachment C for details)
- Milestone reports and other milestone deliverables as outlined in Attachment E and agreed upon by performers and DARPA
- Executive Summary (see Attachment C for details)
- Final Program Report: Performer teams will provide a final report that summarizes all research activities, outcomes, molecular mechanisms, etc., discovered during the program.
    - Proposers are encouraged to submit documentation to support decision making in transition and quality control in future use cases.
    - Proposers are encouraged to present a plan for further testing and development that demonstrates increased adaptability to scopes of stakeholder use cases.
- Any publications, research presentations, or patent applications that result from the research pursued as part of the SMS program.
- Other negotiated deliverables specific to the proposed efforts. These may include registered reports; and/or experimental protocols.

1.10. Detailed Program Schedule

Table 6 *Anticipated Program Schedule and Descriptions of Activities.*

At (or before) End of Month *n*	Description of Activities
Month 0	• Program kick-off (in-person meeting) • **CD1 & CD2** begin (concurrent)
Month 6	• **CD1** concludes • Performers present results of **CD1** to Government teams • IV&V partner begins validation of software • IV&V partner begins ground truth validation experiments
Month 7	• IV&V partners complete experiments, validation of software for **CD1** • IV&V partners compare *in silico* (SIM) and *in vitro* (EXP) datasets (**CD1)** • IV&V partners make appropriate statistical comparisons between performer simulations and "ground truth" data related to **CD1** • Performers and USG teams attend in-person workshop (location TBD) • Technical details of **PTs** furnished to performer teams
Month 8	• Two **PTs** begin for each performer • Performers are encouraged to begin working with the Government and DoD stakeholders to ensure interoperability with their platforms
Month 12	• **CD2** concludes • Performers present **CD2** results to Government teams • IV&V partner begins validation of software • IV&V partner begins ground truth validation experiments
Month 13	• IV&V partners complete experiments, validation of software for **CD2** • IV&V partners compare *in silico* (SIM) and *in vitro* (EXP) datasets (**CD2**) • IV&V partners make appropriate statistical comparisons between performer simulations and "ground truth" data related to **CD2** • Performers and USG teams attend in-person workshop (location TBD)
Month 14	• **PTs** conclude • Performers present results of their PT to Government teams • IV&V partner begins validation of software

	• IV&V partner begins ground truth validation experiments
Month 15	• IV&V partners complete experiments, validation of software for **PTs** • IV&V partners compare *in silico* (SIM) and *in vitro* (EXP) data sets (**PTs**) • IV&V partners make appropriate statistical comparisons between performer simulations and "ground truth" data related to **PTs**
Month 17	• Performers finalize software, data, documentation packages for dissemination, deliver to the Government • Performers complete ensuring software interoperability with USG systems
Month 18	• (Pending DARPA authorization) Performers publish software and data packages to public repositories

1.11. Considerations for Software Development

1.11.1. Platforms to Handle Controlled Unclassified Information (CUI)

Performers will need to operate at the CUI level. This includes prospective individual researchers and all information technology (IT) systems, including but not limited to data analysis, storage, networking and data transfer, cloud, high-performance-computing (HPC), and document systems. Performers will be responsible for ensuring their systems and research adhere to CUI standards (NIST 800-171). Performers may provide their own CUI-certified systems, including laptops, desktops, cloud, HPC, etc. Proposers' Bills of Materials (BOM) may include IT asset requests, provided requests are NIST 800-171 compliant. Solutions may include but are not limited to AWS GovCloud, local servers, etc. Proposed approaches must meet this requirement. See SMS CUI Guide (Attachment F) for more information.

1.11.2. DARPA-Facilitated CUI Compute Options

To assist with the requirement to operate at the CUI level, DARPA can facilitate CUI systems and components thereof.

If desired, DARPA can facilitate access to free shared DoD HPC allocation that is CUI-compatible. Shared allocation for running compute jobs would not be on demand but instead involve jobs being processed after waiting in queues shared across the wider DoD community. Users will be required to use either a DoD CAC or a provided YubiKey, and to undergo online training. If desired, proposals should describe how they would utilize this service.

If desired, DARPA's Information Technology Directorate (ITD) can facilitate the creation of CUI compatible GovCloud Accounts through Amazon Web Services[1] (that is separate from and can interface with DARPA-provided HPC). If proposals would like to utilize this facilitated service, they should include estimates of storage, compute, and any other services (e.g., batch compute, datastores, on-demand parallel clusters for HPC, software development / ML sandboxes, data egress costs/storage transfer, etc.) using the public AWS pricing calculator, choosing their desired AWS GovCloud region. Performers will be responsible for all changes within their provided AWS Account, including, but not limited to, final responsibility for ensuring compliance with NIST 800-171. Should performers utilize this offering, DARPA ITD will have limited consulting availability to provide best practices and advice on environment architecture, CUI compatibility, and other technical and deployment matters.

DARPA and IV&V may also facilitate or provide additional compute, HPC, or other system and components thereof that are CUI-compatible. Performers from selected proposals will have the option of utilizing any such potential systems and components presented during award negotiation

[1] https://aws.amazon.com/compliance/services-in-scope/DoD_CC_SRG/

and initial planning with IV&V. In this similar event, best practices and limited consulting availability may be available. Performers electing to utilize any such potential services will still bear responsibility for ensuring compliance with NIST 800-171.

1.11.3. Notional Simulation Extensions

While SMS will focus on individual *E. coli*, simulation expansion to multiple cells and types may be considered in the future. If considered, DARPA may explore simulations that notionally explore the following properties and higher-order behaviors:

- Interactions between same bacteria such as nutrient depletion and intercellular signaling
- Genetic impacts of quorum sensing molecules
- Interactions between different bacteria such as relative fitness
- Different cells other than bacteria

However, follow-on expansions are NOT solicited in this PS. Any potential expansion may be solicited among SMS performers.

1.12. Dissemination of Research

1.12.1. Publication Approval & Requirements

At this time, DARPA expects much of the work performed under SMS to be unclassified, fundamental research subject to pre-publication review for matters of national security. Information generated that does not clearly identify as "CUI" may still need to undergo review prior to public release. All publications, articles, and scientific presentations will be submitted to DARPA for review and approval 45 days in advance of required submission date, to give time to remove any sensitive information. It is anticipated that workshops and milestone reviews will be used to work towards mutually agreeable plans for review of publication, methods, data, and code prior to release, involving ELSI, IV&V, DARPA, and performers.

To prevent the release of sensitive technical information, certain aspects of the proposed research may be considered CUI if they reveal DoD-specific applications or requirements and may require safeguarding or dissemination controls, pursuant to and consistent with applicable law, regulations, and government-wide policies. SMS CUI guide is in Attachment F. Performers must partition potentially sensitive tasks from nonsensitive research efforts. All performers (prime contractor and subcontractor) desiring public release of project information that may contain CUI as defined above must submit a request for public release from DARPA in accordance with their contractual requirements.

1.12.2. Adding Data and Source Code to Public Repositories

A large amount of raw data is expected to be acquired by performers during program activities (e.g., simulation parameters, genomic & proteomic data). In addition to furnishing these to the Government, performers will also be expected, where appropriate and with approval, to publish these data to public repositories in accordance with applicable law, regulations, and policies. Similarly, in addition to furnishing code and software to the Government and DoD partners and stakeholders, performers may, where appropriate and with approval, publish some or all of the source code to publicly available software repositories.

2. PROGRAM SOLICITATION AUTHORITY

This PS will result in the award of an OT for Prototype Agreement, which can include not only commercially available technologies fueled by commercial or strategic investment but also

concept demonstrations, and development activities that can significantly improve commercial technologies, existing Government-owned capabilities, and/or concepts for broad defense and/or public application(s). The Government reserves the right to award an OT for Prototype Agreement under 10 U.S.C. § 4022, make a partial award, or make no award at all. In all cases, the Government agreements officer shall have sole discretion to select the award agreement type, regardless of agreement type proposed, and to negotiate all agreement terms and conditions with selected proposers. The OT agreement will not require cost sharing unless the proposer is a traditional defense contractor who is not working with a non-traditional defense contractor to a significant extent.

2.1. PS Procedure

In response to this solicitation, and after verifying eligibility, proposers are asked to submit an 8-page abstract as described in Section *4.2*. This process allows DARPA to ascertain (1) whether the proposers understand the key challenges of the SMS program and (2) whether they are capable of executing their proposed concept. Specific evaluation criteria used by DARPA to make the assessment can be found in Section *4.3*. If DARPA finds that both of these conditions are met, it may invite the proposer to submit an Oral Proposal Package (OPP) as described in Section *4.4*, and participate in an oral presentation to DARPA, where the proposed technical solution will be evaluated. After the oral presentations, DARPA will decide which proposers will be selected to participate in the program. Specific evaluation criteria used to make the assessment can be found in Section *4.6*. At the end of SMS, DARPA anticipates modifying the existing Prototype OT agreement to include follow on Periods of Performance (PoP). The Government will not pay proposers responding to this PS for the costs associated with abstract submissions, OPP preparation, oral presentations, or future proposal development.

DARPA will use the following process to facilitate the SMS source selection:

a. **Industry Day (Optional):** The Program Manager held an Industry Day where he briefly described the program and its goals and solicit questions from the audience in real time. Where possible, the Government provided answers in real time, and a comprehensive list of questions and answers will be provided afterward via a Question and Answer (Q&A) document. Participation in the Industry Day was optional and was not a requirement for proposers seeking to submit an abstract.

b. **Question and Answer (Q&A) (Informational Only):** DARPA hosted a question and answer (Q&A) session during the SMS Industry Day and will post a consolidated Q&A document. The Q&A document will be available online at https://www.darpa.mil/work-with-us/opportunities. Questions can be sent to SMS@darpa.mil prior to the Questions Due Date (*above*). DARPA will respond to any relevant and/or PS clarification question(s) prior to the final abstract due date (*above*) and post consolidated Q&As at the DARPA Opportunities page (https://www.darpa.mil/work-with-us/opportunities).

c. **Abstracts (Required)**: Abstracts shall be submitted as specified in Section *4.2* of this PS. The Government will review all submitted abstracts for technical comprehension and ability (see Section *4.3*). Selected proposers will be invited to provide an OPP and participate in an oral presentation (see Section *4.4*) to the Government. Note that proposers must submit an abstract(s) in response to this solicitation to be considered for participation in the SMS program. Proposers will not be invited to submit an OPP, provide an oral presentation, or be included in any further progression of the program without participating in the abstract phase of the solicitation.

d. **Oral Proposal Package (OPP) & Oral Presentation (Required if selected)**: Oral presentations are anticipated to take place approximately five weeks after notification of abstract decision, with OPP content due to DARPA approximately three weeks after notification of abstract decision. OPP content and format, including technical clarification document, model OT, and others, is detailed in Section *4.4*, however the final requirements, to include templates, submittal instructions for OPPs, and proposed presentation dates for oral presentations will be provided in the invitation to submit an OPP and participate in an oral presentation. The Government will review all OPPs (see Section *4.5*), which will not be made public or provided to other proposers. For SMS, proposers must only propose an OT for Prototype with fixed payable milestones. (**Note** – Milestones represent a completed event. Milestone schedule is based on key observable events in the critical path to accomplish program objectives. Payments are triggered by successful performance of observable technical events. Fixed payable milestones are payments based on successful completion of the milestone accomplishments agreed to in the milestone plan. Proposals may suggest modifications or additions to the Schedule of Milestones and Payments; please note that suggested edits may not be accepted by DARPA. A Schedule of Milestones and Payments is included as Attachment E.)

e. **Program Length (18 months):** DARPA will review OPPs and oral presentations to determine which proposed solutions sufficiently meet the evaluation criteria stated in Section *4.6*. Upon favorable review, and subject to the availability of funds, the Government may award an OT for Prototype under 10 U.S.C. § 4022 with fixed, payable milestones for program selectees.

3. ELIGIBILTY INFORMATION

3.1. Eligible Applicants

3.1.1. Federally Funded Research and Development Centers (FFRDCs) and Government Entities

For Federally Funded Research and Development Centers (FFRDCs), University Affiliated Research Centers, and Government entities interested in participating in the SMS program or proposing to this PS should first contact the Agency. Point of Contact (POC) listed in the Overview section prior to the proposal due date to discuss eligibility.

3.1.2. Other Applicants

Non-U.S. organizations and/or individuals may participate to the extent that such participants comply with any necessary nondisclosure agreements, security regulations, export control laws, and other governing statutes applicable under the circumstances.

3.2. Organizational Conflicts of Interest (OCI)

An organization cannot simultaneously provide scientific, engineering, technical assistance (SETA), advisory and assistance services (A&AS), or similar support to DARPA and also be a performer on a DARPA research program.

If a prospective proposer believes a conflict of interest exists or may exist (whether organizational or otherwise) or has questions on what constitutes a conflict of interest, the proposer must send their contact information and a summary of the potential conflict via the specific e-mail address identified in this PS before time and effort are expended in preparing any

submission documentation

4. GUIDELINES FOR ABSTRACTS, ORAL PROPOSAL PACKAGE (OPP), AND ORAL PRESENTATIONS

4.1. General Guidelines

a. Do not include elaborate brochures; only include information relevant to the submission requirements or evaluation criteria.
b. Use of a diagram(s) or figure(s) to depict the essence of the proposed solution is permitted.
c. All abstracts and oral presentations shall be unclassified.
d. Proposers are responsible for clearly identifying proprietary information. Submissions containing proprietary information must have the cover page and each page containing such information clearly marked with a label such as "Proprietary" or "Company Proprietary." **NOTE: "Confidential" is a classification marking used to control the dissemination of U.S. Government National Security Information as dictated in Executive Order 13526 and should not be used to identify proprietary business information.**
e. Questions regarding abstracts can be sent to SMS@darpa.mil (see *above* for Question Due Date).
f. Submit Abstracts to SMS@darpa.mil (see *above* for Abstract Due Date).
g. Submissions sent through other mediums, channels, or after the prescribed Program Solicitation deadlines (see *above*) will not be considered, reviewed, or evaluated.
h. Proposers providing abstracts that are not invited to an oral presentation will be notified in writing as soon as practical.

4.1.1. Technical Guidelines

Proposers are encouraged to provide a technical and programmatic strategy that conforms to the entire program schedule and presents an aggressive plan to fully address all program goals, metrics, milestones, and deliverables, and therefore are encouraged to:

- Describe their approaches for Simulate & Predict and Measure & Inform, and how, taken together, those approaches will meet program goals (see *1.4*), fulfill CD and PT (see *1.5*), while meeting program CUI requirements (see *1.11*)
- Describe credible plans to simulate two use cases, as well as higher-order properties and behaviors (see *1.4.2*); and to meet program metrics (see *1.6*), including information about how to establish simulations' equivalence with ground truth information (see *1.6.2*)
- Include credible plans to have continuous and deliberate interactions with IV&V partners and ELSI that meet program goals (see *1.7* and *1.8*)
- Detail key risks and describe risk mitigations
- Describe credible plans to meet program data, simulation, and other deliverables (see *1.9*)

Proposers may refer to Attachment G for suggested technical content when preparing their submission documents. Attachment G is not meant as evaluation criteria for any source selection step, but instead is meant as a single list to help proposers include technical information.

4.1.2. Programmatic and Organizational Guidelines

In addition, proposers should:

- Describe structures and activities to communicate and engage with the Government sponsor, Government stakeholders, and relevant prospective regulatory agencies to facilitate

a feasible path towards transition and potential commercialization of the technology.

- Provide a technical and programmatic strategy that emphasizes how contributions to research will be durable, repeatable, and extendable, including:
    - Code documentation, portability, requirements/dependencies, ability to install and run on IV&V and sponsor systems
    - Experimental quality control, metadata and contextual information, including media, measurement techniques, experimental contexts (e.g., experiments on single proteins vs those run on those proteins in cells)
    - Anticipated contributions publicly available databases (e.g., EcoCyc, GenoBase, KBase).
- Structure themselves to conform to best practices for all simulation code, including version control, security, collaboration, documentation, programming language specifications, providing example data formats, reproducible containers, etc., to best facilitate IV&V and transition.
- Provide a clear understanding of the cost, risk, and organizational expertise to be used within each proposed effort. If selected for Oral Presentation, proposers must include detailed pricing and a Task Description Document (TDD) for the effort (see model OT).
- Provide a task structure that is consistent across the proposed schedule, TDD, and cost volume. A target start date of approximately six months after Oral Presentations (see *above*) may be assumed for planning and budgeting purposes.
- Include the following meetings and travel in the proposed schedule and costs:
    - Proposers should budget for attending the two (2) in-person workshops, which will function as semi-annual technical and programmatic reviews. These will foster collaboration between teams and disseminate program developments.
        - For budget planning purposes, proposers should assume locations split between the East and West Coasts of the United States and can plan for two (2) two-day meetings.
    - Proposers should budget for at least two (2) meetings to engage with the Government IV&V partners and to support IV&V assessments, to be coordinated between performers and IV&V at or following program kickoff. Meetings are encouraged to be in-person but may be virtual or hybrid.
    - Regular teleconference meetings (e.g., monthly) will be scheduled with the Government Team for progress reporting as well as problem identification and mitigation. Performers should provide financial and technical research updates, including any Simulate and Predict updates, Measure and Inform updates, and ELSI updates. These reports should be in the form of a standardized slide presentation provided to DARPA and discussed with the program management team via teleconference. Length and detail level should be at the discretion of the Program Manager.

Proposers should anticipate at least one site visit by the DARPA Program Manager during which they will have the opportunity to demonstrate progress towards agreed-upon milestones. This is in addition to PI meetings, demonstrations, and workshops.

4.1.3. Teaming

Proposers are responsible for assembling a complete team that has technical expertise, capabilities, and facilities to address all requirements of the program. This includes significant experience in the research and development of computational cellular simulation, as well as significant experience and expertise in *in vitro* experimentation for data collection about *E. coli*, as

well as in any computational techniques or advances mentioned in this solicitation (e.g., physics-based modeling).

Proposers should demonstrate flexibility to address pressure tests and use cases. Teams may provide information relevant to use cases, CDs, and PTs (e.g., strengths, weaknesses, preferences, areas of expertise). If desired, teams may identify SMEs to meet pressure tests or use cases and if so identifying, should include corresponding proposed options in their proposals. Proposers must identify team members or vendor sources required to complete CDs, PTs, and any other milestones where applicable.

All teams are encouraged to identify a Project Manager to serve as the primary point of contact to communicate with the DARPA Program Manager and Agreements Officer Representative, and Technical Representatives (TR), coordinate effort across performer team, organize regular performer meetings or discussions, facilitate data sharing, and ensure timely completion of milestones and deliverables (see *1.9*). For teams that are not physically co-located, proposers must articulate how logistical challenges will be overcome to ensure smooth collaboration and an integrated work product.

4.1.4. Data Sharing and Associate Performer Agreements (APA)

DARPA anticipates that a large amount of data will be generated under this program by each performer team. Data analysis and modeling will be strengthened by compiling and integrating information across Simulate and Predict, and Measure and Inform areas, as well as shared from IV&V and/or other government partners. Therefore, proposals must include the description of a plan to share data between computational and wet lab research thrusts, and to share data with IV&V. As needed, data sharing plans to facilitate exchange will then be formalized in an Associate Performer Agreement (APA), to be included in the agreement awarded. As needed to achieve the goals of the program, DARPA may also facilitate formalized APAs for performers sharing data with other performers. Performers will be encouraged to share data externally with the broader research community, after any sensitive information or capabilities are controlled per security regulations and guidance, and performers may include plans for external data sharing in the milestones, metrics, and deliverables.

4.2. Abstract Content

Proposers must submit an abstract to be considered for an award. Abstracts should not exceed eight (8) single-sided written pages using 12-point Times New Roman font with 1" margins all around. Abstracts that do not conform to these requirements may not be evaluated. The submitted abstract must consist of the following information:

a. ***Abstract Summary Slide:*** See Attachment A: Abstract Summary Slide Template. This summary slide does not count against the eight (8) written page limit.

b. ***Title page:*** Proposer Name, Title, Date, Point of Contact (POC) Name, E-Mail Address, Phone, and Address. The title page must include a statement that the terms of the model OT agreement (Attachment C Model Research Other Transaction (OT)) are acceptable to the proposing organization without changes. The proposer shall also include a statement on the title page that no people on the proposer's team(s) work for DARPA as SETA, A&AS, or similar support services, as DARPA has a policy prohibiting such people from working as a technical performer (see Section *3.2*). The title page does not count against the eight (8) written page limit.

c. ***Executive Summary*:** Provide a brief summary of the technical problem the SMS program seeks to solve. This summary shall be stated in the proposer's own words without any "copy

and paste" of this solicitation. The goal is for the proposer to demonstrate clear and quantitative understanding of the purpose and goals of the SMS program. The Executive Summary shall be no more than one (1) page and is not included in the eight (8) written page limit.

d. ***Proposed Approach*:** Provide a summary of the proposed technical approach to achieve SMS's goals and metrics. Methods to address the challenges (see below) must be provided. Highlight the novelty and uniqueness of the proposed work with respect to prior efforts. The Proposed Approach is included in the eight (8) written page limit.

e. ***Technology Challenges*:** Identify specific challenges associated with this proposed approach. The proposer should include what they think the primary risks are to successful development in the SMS program. In other words, what are the critical challenges related to the proposed approach? The Technology Challenges is included in the eight (8) written page limit.

f. ***Technical Ability*:** Detail why the proposer believes their team has the ability to be successful at achieving the program goals, if selected to participate in SMS. The proposer may include past experience, organizational capabilities, team members' qualifications, or anything else that demonstrates competence in designing and demonstrating desired SMS outcomes. The Technical Ability is included in the eight (8) written page limit.

g. ***Estimated Cost:*** Include the estimated total labor cost, and estimated materials and other direct costs (ODCs; e.g., equipment, materials, travel, tuition). Total costs must not exceed $8M of what would be Government funding. This may be presented as a narrative or table (less than 0.5 pages) and is not included in the eight (8) written page limit.

h. ***References:*** Provide a list of citations, references, or end notes. References should be included as an appendix to the abstract and are not included in the eight (8) written page limit.

4.3. Abstracts: Process & Basis of Evaluation

Abstracts will be evaluated by DARPA using the evaluation criteria listed below in descending order of importance, and not against other abstracts submitted in response to this PS. As stated above, proposers are required to submit an abstract for evaluation by DARPA to be considered for any subsequent award. DARPA will respond to the 8-page abstract with a statement as to whether or not DARPA requests submission of an Oral Proposal Package. Upon review of abstracts, the Government may elect to invite all, some, or none of the proposers to submit an OPP and participate in oral presentations. *Only abstract proposers invited by DARPA to submit an OPP and participate in oral presentations are eligible to provide one.*

- ***Technical Comprehension:*** The proposed technical understanding is accurate, proposed approach is clearly described, and key technical challenges and risks are identified. The proposed approach must include plans to address CDs and PTs as they relate to use cases and relevant *E. coli* properties and behaviors, and method(s) to establish equivalence with IV&V experiments. Technical approaches to challenges are supported by brief calculations or physical estimates where possible.
- ***Technical Ability:*** The proposer's team and organization demonstrate the ability to achieve the goals of the program.
- ***Cost Rough Order of Magnitude (ROM):*** The proposed ROM is reasonable, realistic, and affordable for the technical approach and accurately reflect the technical goals and objectives of the Program Solicitation.

4.4. Oral Proposal Package

If DARPA expresses interest in an oral presentation, the proposers will be asked to provide further details on its proposed solution. Specific instructions (including content submission guidelines) will be provided in the invitation to participate. In the event the instructions in the invitation to submit an OPP and participate in an oral presentation deviate from the following list of expectations, the instructions in the invitation to participate take precedence.

Oral presentations will be over the course of 1-2 days (anticipated eight weeks after abstracts are due, see *above*) in the Washington, DC metro area. Virtual presentations will be allowed where in-person attendance is not possible. Each oral presentation will be 60 minutes total; it is anticipated that each will consist of up to a 40-minute presentation followed by the remainder for Q&A. Oral Proposal Packages must be **submitted in advance** of providing presentations (see deadline *above*). Oral Proposal Packages must include:

a. **Title page:** Proposer Name, Title, Date, Point of Contact (POC) Name, E-Mail Address, Phone, and Address. (The title page does not need to be briefed).

   - The proposer shall also include a statement on the title page that no people on the proposer's team(s) work for DARPA as SETA, A&AS, or similar support services on an active contract or subcontract (including those awarded through DARPA agents), as; DARPA policy prohibits support contractor individuals and entities from concurrently working as research and development performers (see Section *3.2*).
   - The proposer shall include a statement that identifies and substantiates which of the following condition(s) are met to permit use of OTs for Prototypes in accordance with 10 U.S.C. § 4022(d)(1): (A) There is at least one nontraditional defense contractor or nonprofit research institution participating to a significant extent in the prototype project; (B) All significant participants in the transaction other than the Federal Government are small businesses (15 U.S.C. § 638) or nontraditional defense contractors; (C) At least one third of the total cost of the prototype project is to be paid out of funds provided by sources other than the Federal Government; or (D) The senior procurement executive for the agency determines in writing that exceptional circumstances justify the use of a transaction that provides for innovative business arrangements or structures that would not be feasible or appropriate under a contract, or would provide an opportunity to expand the defense supply base in a manner that would not be practical or feasible under a contract.

b. **Oral Proposal presentations** should include the following information, addressed in any order: **(up to 40 minutes, slides submitted in advance)**:

   - Facilities and teaming/personnel qualification – Describe how past performance and qualifications of key personnel will contribute to the technical approach. Identify and explain efforts of similar scope and complexity.
   - SMS technical approach – Provide detailed description of innovative claims and how they will achieve SMS objectives and metrics.
   - Detailed risks and mitigation plan – Demonstrate that you have clearly considered the technical risks and have well thought out mitigation plans.
   - Proposed schedule
   - Any pertinent updates from the abstract submission, including technical, budget, etc.

- Examples of past performance or projects in the relevant (i.e. Simulate & Predict, Measure & Inform) technical domain(s), and regulatory and translation experience (if any) following prototype development. Aspects to consider including would be, but are not limited to, highlighting key personnel who will work on the program, providing examples of past performance or technical projects, and/or demonstrating capabilities relevant to SMS goals.

c. **Model OT for Prototype Agreement*:*** Proposers must complete and submit the Model Other Transaction (OT) for Prototype provided as Attachment C as part of the Oral Proposal presentation package. DARPA has provided the model OT in order to expedite the negotiation and award process. The Model Other Transaction (OT) for Prototype is representative of the terms and conditions that DARPA intends to award for SMS includes the following eight (8) attachments:

    - Attachment 1 Task Description Document
    - Attachment 2 Report Requirements
    - Attachment 3 Schedule of Milestones and Payments
    - Attachment 4 Agreements Officer's Representative Appointment Memo
    - Attachment 5 Property/Equipment
    - Attachment 6 Performer Attestation
    - Attachment 7 Associate Performer Agreement
    - Attachment 8 Certifications for Agreement

    It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For proposals that contain cost share, the proposer should provide sufficient rationale as to the appropriateness of the cost share arrangement relative to the objectives of the proposed solution (e.g. high likelihood of commercial application, etc.). Proposers may suggest edits to the model OT for consideration by DARPA and provide a copy of the model OT with tracked changes as part of their proposal package. Please note that suggested edits may not be accepted by DARPA. The Government reserves the right to remove a proposal from award consideration should the parties fail to reach agreement on OT award terms and conditions. If edits to the model OT are not provided as part of the proposal package, DARPA assumes that the proposer has reviewed and accepted the award terms and conditions to which they may have to adhere and the sample OT agreement provided as an attachment, indicating agreement (in principle) with the listed terms and conditions applicable to the specific award instrument. DARPA explicitly reserves the right to terminate awards if negotiations are not completed in a timely manner.

d. **Determination of Process Improvements, Creating Value via OT for Prototype Agreement Vehicle:** Proposers are required to provide answers to all of the following questions as part of the Oral Presentation Package. (Please note these will not be presented during the oral presentation and will be reviewed whether presented or not. Further, the answers to the questions are not subject to any oral presentation evaluation criteria.) *Questions to be answered are as follows*:

1. Please provide your understanding of current technology in this space, and how it has informed or influenced your proposed technical solution.
2. How does your proposed solution deliver increased capability beyond what is possible today?
3. How would your proposed solution, if successful, enable federal entities to do that they cannot already?
    a. How much time and money could the DoD / Federal Government save when compared to the current state of technology?
    b. What future value does this technology offer to the DoD / Federal Government?
    c. What commercial best practices or processes do you plan to instantiate to deliver value to the Government?
4. How would your proposed solution, if successful, enable the commercial markets to do that they cannot already?
    a. What future value does this technology offer to the commercial sector?
    b. Is your solution disruptive to the market, or does it provide incremental improvements to current practices?
5. Detail the technical risks in your proposal to be solved under the DARPA program. How does DARPA engaging in this program accelerate the timeline for value, schedule, technical debt, and transition to commercial or DoD marketplaces?

Proposers are free to provide further detail outside of the answers to the above questions as to why and how an OT allows for the Government to realize cost savings and thereby create added value.

e. **Cost Spreadsheet:** Proposers must fully complete Attachment D Cost Spreadsheet for SMS. Provide rough order of magnitude estimates for the technical efforts described in Section *1* above.

f. **Technical Clarification Document (TCD):** Proposers will submit a technical clarification document no longer than six pages in length as part of the OPP. The TCD should explain in detail responses to the DARPA team's ask(s) for technical clarification, which will be provided with or shortly after abstract decisions. This TCD should also include a risk assessment matrix where the probability and impact of the most important risks associated with the critical path are each assigned a number (Probability: 1-5; 1=low, 5=high; Impact: 1-5; 1=low, 5=high). A brief justification for each technical risk will be included.

4.5. Oral Proposal Evaluation Criteria

OPP / oral presentation evaluation criteria are listed in descending order of importance. Individual presentations will be evaluated against the evaluation criteria described below:

- **Technical Approach:** The proposed technical approach is innovative, feasible, achievable, and complete. The proposal demonstrates an innovative yet feasible approach to address the identified technical risks and challenges to meet program metrics.
- **Potential Contribution and Relevance to the DARPA Mission:** The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. Any proposed intellectual property restrictions will not significantly impact the Government's ability to transition the technology (see Section *5.3*).

- **Relevant Qualifications:** Personnel and/or company demonstrate the ability to meet the technical goals of the program, provide examples of past performance or projects in the relevant technical domain(s) (i.e. Simulate & Predict, Measure & Inform), and demonstrate capability to navigate regulatory and translation challenges anticipated in the program.
- **Budget:** The proposed solution is reasonable, realistic, and practical use of the allotted funding.

The Government reserves the right to record presentations. The Government will rely on information provided in the oral presentation, attachments, and Q&A session as basis for evaluation. Oral presentation will be evaluated by the SMS Program Manager with support from SETA subject matter experts (SMEs). Oral presentation slides that are not orally presented by the proposer within the allocated time interval will not be evaluated. There is no formal page limit on the number of slides or attachments.

After completing evaluation of oral presentations, DARPA will: 1) negotiate an 18-month award for SMS; or 2) inform the proposer that its proposed concept/solution is not of continued interest to the Government, and they are no longer considered for the program. If DARPA does not intend to issue an award for future PoP efforts to a proposer, DARPA will provide feedback to the proposer regarding the rationale for this decision.

4.6. Review and Selection Process

DARPA's policy is to ensure impartial, equitable, and comprehensive proposal evaluations based on the evaluation criteria listed above and to select the source (or sources) whose proposal meets the Government's technical, policy, and programmatic goals. DARPA will conduct a review of each conforming abstract and OPP. All evaluations will be based solely on the evaluation criteria in Section 4. Using the evaluation criteria, the Government will evaluate each abstract and OPP in its entirety, documenting the strengths and weaknesses relative to the evaluation criteria. Based on the identified strengths and weaknesses, DARPA will determine whether an abstract or OPP is selectable. DARPA will not evaluate abstracts and OPPs against each other during the scientific review process, but rather evaluate the abstracts and OPPs on their own merit to determine how well the proposal meets the criteria stated in this PS. DARPA will make an award to a proposer whose abstract and OPP are determined to be selectable by the Government, consistent with instructions and evaluation criteria specified in the PS, and based on availability of funding. Given the limited funding available, not all proposals considered selectable may be selected for a potential award. For the purposes of this proposal evaluation process, DARPA defines a "selectable" and "non-selectable" abstract or OPP as follows:

Selectable: A selectable abstract or OPP is one that the Government has evaluated against the evaluation criteria listed in the PS, and the positive aspects outweigh the negative aspects.

Non-Selectable: An abstract or OPP is considered non-selectable when the Government has evaluated it against the evaluation criteria listed in the PS, and the positive aspects do not outweigh the negative aspects.

5. AWARDS

5.1. General Guidelines

Upon favorable review of the proposal and subject to the availability of funds, the Government may choose to negotiate an award an OT for Prototype Agreement. The Government Agreements Officer reserves the right to negotiate directly with the proposer on the terms and conditions prior to execution of the resulting OT agreement, including payment terms, and will execute the agreement on behalf of the Government. Be advised, only a Government Agreements Officer has the authority to enter into, or modify, a binding agreement on behalf of the United States Government. In order to receive an award:

a. Proposers must have a Unique Entity Identifier (UEI) number and must register in the System for Award Management (SAM). Proposers are advised to commence SAM registration upon notification of entry of the competition.

b. Awardees will be required to submit invoices for payment electronically via the Wide Area Work Flow (WAWF) module in the Procurement Integrated Enterprise Environment at https://piee.eb.mil/, unless an exception applies. Registration in PIEE is required prior to any award under this PS. For assistance with PIEE, please contact 866-618-5988 or DARPAInvoices@DARPA.mil.

c. Proposers must be determined to be responsible by the Agreements Officer and must not be suspended or debarred from award by the Federal Government nor be prohibited by Presidential Executive Order and/or law from receiving an award.

d. Being asked to submit a proposal does not guarantee that a proposer will receive an award. The Government reserves the right not to make an award.

5.2. Competition Sensitive Information

DARPA policy is to treat all submissions as competition sensitive, and to disclose their contents only for the purpose of evaluation. Restrictive notices notwithstanding, during the evaluation process, submissions may be handled by support contractors for administrative purposes and/or to assist with technical evaluation. All DARPA support contractors performing this role are expressly prohibited from performing DARPA sponsored technical research and are bound by appropriate nondisclosure agreements. Input on technical aspects of the proposals may be solicited by DARPA from non-Government consultants/experts who are strictly bound by the appropriate non-disclosure requirements.

5.3. Intellectual Property/ Data Rights

SMS will produce simulation software and technical data that will be furnished to stakeholders including the DoD. The Government expects unlimited rights for the technology and data developed and/or generated under the SMS program but is open to flexible intellectual property (IP) proposals from performers that are advantageous to the Government. See Section *4.5*.

Proposers responding to this solicitation shall appropriately identify any potential restrictions on the Government's use of any intellectual property furnished by the proposer. This includes both Noncommercial Items and Commercial Items. Proposers are encouraged to identify these restrictions in a format similar to the table depicted below. If no restrictions are intended, then the proposer should state "NONE."

Technical Data, Computer Software To be Furnished with Restriction	Summary of Intended Use in the Conduct of the Research	Basis for Assertion	Asserted Rights Category	Name of Person Asserting Restrictions
(LIST)	(NARRATIVE)	(LIST)	(LIST)	(LIST)

5.4. Procurement Integrity Act (PIA)

All awards under this PS shall be treated as Federal Agency procurements for purpose of 41 U.S.C. Chapter 21. Accordingly, the PS competitive solicitation process and awards made thereof must adhere to the ethical standards required by the Procurement Integrity Act.

6. REFERENCES

Ghatak, S. K. (2019). The y-ome defines the 35% of Escherichia coli genes that lack experimental evidence of function. *Nucleic acids research, 47(5)*, 2446-2454.

Jumper, J. R. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature*, 583-589.

Passi, A. J.-B.-C. (2021). Genome-scale metabolic modeling enables in-depth understanding of big data. *Metabolites 12, no. 1* .

Shu, H. J. (2021). Modeling gene regulatory networks using neural network architectures. *Nature Computational Science 1, no. 7*, 491-501.

Thornburg, Z. R. (2022). Fundamental behaviors emerge from simulations of a living minimal cell. *Cell 185, no. 2*, 345-360.

Yang, W. Z. (2014). Combining high-throughput phenotyping and genome-wide association studies to reveal natural genetic variation in rice. *Nature communications 5, no. 1* .