DARPA-PA-25-06 PROtein Sequencing (PROSE)

Question and Answer (Q&A) Document

Version 2, 7/22/2025

1. The goal of the PROSE program is stated as "full protein sequencing identification without a reference". Should this be interpreted as DARPA wants to read every amino acid not just a sufficient number to ID the protein?
    a. There is no requirement that the system and associated algorithms "ID" the protein. The PA calls for systems to read every letter in sequence, with a minimum read length, at a desired single letter accuracy and overall system throughput.
2. Can a proposer use a reference in the letter calling algorithm as part of development? It would still be de novo and unknown but the reference would be part of the AA calling algorithm.
    a. It is anticipated that letter calling algorithms could require training with known sequences and this is within the scope of the program.
3. The program goal to read sequences of at least 300 amino acids in length suggests full length protein sequencing vs peptide based methods. Are peptide based methods acceptable if they can achieve a high level of amino acid coverage per protein?
    a. The program is agnostic to how the sequence is assembled and is open to many approaches to how the sequence length is achieved. However, approaches must demonstrate a path to achieving all metrics, including accuracy and throughput metrics.
4. Is the government open to approaches that include non-nanopore based methods?
    a. The program is open to non-nanopore based methods.
5. When does DARPA expect to provided feedback on abstract submissions?
    a. DARPA anticipates providing responses and feedback on abstracts within approximately 14 days after submission.
6. When will awards be made for Phase I?
    a. Awards are anticipated for early calendar year 2026.
7. How are the Phase II performers chosen?
    a. Phase I performers will be moved to Phase II based primarily on the government's assessment of the phase-ending design review. However, progress towards metrics and general performance will be considered.
8. How are the protein sequences for the 50 and 100-letter milestone tests decided? Are they chosen by the performer, DARPA PM/staff, or a combination?
    a. Protein sequences will be chosen by the government team. The government team will take inputs from performers and the IV&V team, but reserves the right to determine all sequences.
9. Is there anything off-limits or not recommended to include in the budget (e.g. salaries, equipment, consumables)? Are there limits?

a. The proposed budget should include all direct costs required to complete the effort as proposed and outlined in the TDD, as well as all applicable indirect costs. As noted in the PA, fee/profit should not be included when resource share is proposed.

10. Is there a standard budget template that will be provided?
    a. Yes, please see Attachments D & E posted with the PROSE Program Announcement. As noted, while Attachment D & E are **required** to be submitted with a full proposal, proposers **may** include additional spreadsheets in proposer format if beneficial for the government review, understanding, and mapping of the proposed price.
11. At what stage does DARPA typically connect performers with DARPA venture partners?
    a. CSO can assist with connecting performers at any time within the program timeline, at the discretion of the government team.
12. Is 'single molecule' protein sequencing one of the goals?
    a. The program is agnostic to the technical approach. However, proposed approaches must demonstrate a path to achieving all metrics, including accuracy and throughput metrics.
13. What is the expected protein concentration in the IV&V sample and what are the purity metrics, in particular when it comes to PTMs?
    a. There are no sample purity metrics. At demonstrations, samples will be provided to the teams at a reasonable concentration and purity, within the limits of IV&V synthesis capabilities. The IV&V team will work with performers throughout the program to produce well characterized test samples that are amenable to the performer's approach.
14. Do I need to be a US company to be eligible to apply?
    a. Per the PA, all responsible sources, to include non-U.S. source, may submit a proposal.
    b. 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
15. It was mentioned at Industry Day that sample preparation is outside the scope of PROSE. Does this mean test proteins can be synthesized with any required adaptor(s) pre-attached?
    a. Adaptor attachment is the sole responsibility of the performer team. Proposals must include a detailed description of the approach required to attach adapters. The IV&V team will develop test proteins that mimic those found in nature to the greatest extent practical.
16. What is the program size and any guidelines for budget size per project proposal submission?
    a. There is no target price for a PROSE project or effort. A proposer's price volume should include all costs commensurate with the technical effort proposed and outlined in the TDD.
17. Is it a correct assumption that the effort done by the IV&V team does not need to be budgeted in the project proposal? So proposer team can assume the contribution is included or does it need to be scoped and separate budget prepared for the IV&V team work?
    a. The IV&V work described in the PA, and any costs associated with it, will be funded by DARPA directly. Proposers do not need to budget for IV&V activities.

18. Can DARPA provide more details on potential DoD or U.S. Government customers?
    a. There is no specific customer in mind. There are many potential applications/transition of PROSE technology throughout the DOD and commercial ecosystems.
19. Can a proposal include fee when zero resource share is proposed.
    a. Yes. Fee can be included when zero resource share is proposed and the government is funding 100% of the effort.
20. Is the accuracy metric based on population or on single molecule?
    a. The accuracy metric is single letter read accuracy. The program is agnostic to the approach for achieving single letter accuracy, as long as all program metrics can be achieved.
21. How is proposal review done? Is it internal or external?
    a. Proposals are reviewed by government employees in accordance with the evaluation factors outlined in the PA. For more information on DARPA's Scientific Review Process please see the link in Section II, Evaluation Criteria of the PA.
22. How is the funding for each phase allocated?
    a. Proposers should include cost for each Phase of PROSE commensurate with the technical effort proposed and outlined in the TDD.
23. For companies that have completed a related SBIR project. Would you consider using a SBIR Phrase III contracting vehicle?
    a. Per the PA, OT for research contracts will be contracting vehicle for this solicitation.
24. I am also a US citizen, MIT graduate, and I am trying to spin off a US/International start-up regarding our technology. Do I need to apply based on "this" company, which is very much not so established YET?
    a. DARPA cannot advise on which entity should submit as the Prime to PROSE; the decision is a business case specific to each team. Per the PA, all responsible sources are eligible. Therefore, all teams should carefully consider which entity offers the strongest technical expertise to lead the development and execution of the highest-quality technical approach aligned with PROSE program goals, and is best equipped to handle all administrative requirements, including SAM.gov and WAWF registration, finance management, reporting, and compliance.
25. If a proposer team includes university research labs with foreign nationals, what would be the timeline for foreign disclosures?
    a. While there's no specific required disclosure timeline, it's highly recommended to address foreign national involvement as early as possible, ideally during the proposal stage. The full PROSE proposal must demonstrate compliance with the PROSE CUI Guide, which could include addressing this issue. Proactive disclosure allows DARPA and the proposer to work together to identify and mitigate any potential security or compliance concerns before award, preventing delays or impacting performance.
26. What is "special" about the designation "Fundamental" regarding research or proposal. I didn't "get" it...

a. Fundamental research means basic and applied research in science and engineering, the results of which ordinarily are published and shared broadly within the scientific community, as distinguished from proprietary research and from industrial development, design, production, and product utilization, the results of which ordinarily are restricted for proprietary or national security reasons. Fundamental research products (i.e., research results, publications) are to remain unrestricted to the maximum extent possible. Given the nature of the PROSE program as outlined in the PA and CUI Guide, there may be aspects of PROSE that could be considered fundamental and non-fundamental.

27. What is a "microsystem" in the context of PROSE
    a. A microsystem is a miniaturized system or device that integrates various components, such as sensors, actuators and control circuits, on a single substrate to perform protein sequencing.
28. Will the IV&V team provide all molecules within the program?
    a. The IV&V team will prepare a library of molecules to be used at demonstrations ONLY. It is anticipated that performer teams will procure their own samples for system and algorithm development.
29. Accuracy is defined as "single letter". Are there constraints on this metric?
    a. Each letter read by the sequencer must be accompanied by a read accuracy measurement. The read accuracy is for that letter ONLY and is not an average across many letters in the sequence.
30. Will IV&V be providing letters for the demos in Phase 1? If so, when will they be distributed?
    a. The IV&V team will be providing samples for Phase 1 and Phase 2. They will be distributed to teams with sufficient time for performers to train and test their model before the milestones are due.
31. If non-US persons are already involved with the project, should we cut them out of the proposal? How has this been managed in the past?
    a. Non-US persons are not restricted from participating on the proposer's team.
32. How much of Phase 2 (e.g. budget, work plan and microsystem design) needs to be included in the abstract and final proposal due in August?
    a. Proposers should include the same level of detail for Phase 1 and Phase 2 and clearly communicate their work plans and design plans at each stage of the program referencing the program announcement and all relevant attachments and templates. For Phase 2, Proposers should include pricing assumptions used to derive the costs, such as escalation and complexity factors.
33. For early stage startups, is evaluation of commercial potential based solely on our commercialization plan, or does VC investment contribute?
    a. The commercialization plan should provide a high-level overview of the commercialization strategy to ensure that the proposers have laid out a commercial path for the technology developed during the program, including but not limited to VC investment. Please provide any relevant information that supports the path laid

out in the proposal and refer to the PA for additional specifics. We also acknowledge that this will be a living document, and the initial strategy outlined in the proposal is a starting point that will develop throughout the course of the program.

34. Can an entity submit more than one abstract?
    a. While there is no restriction on the number of abstracts an organization may submit, each submission should contain a unique technical approach. Organizations are encouraged to prioritize quality and originality, as DARPA is seeking truly revolutionary solutions.