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Advancing Cell-Free Manufacturing: Challenges in Scale-up and Automation Workshop Report

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Abstract

The National Institute of Standards and Technology (NIST) and the National Center for Advancing Translational Sciences (NCATS) convened the workshop Advancing Cell-free Manufacturing: Challenges in Scale-up and Automation in Rockville, Maryland in February 2024. This workshop brought together over fifty participants, representing the interests and needs of stakeholders in academic, industrial, and government settings. Together, through various plenary discussions, case studies, and working groups, participants broadly surveyed the field and focused on identifying both near-term and long-term needs to support cell-free expression systems. This report synthesizes the workshop discussion and presents actionable recommendations aimed at removing the remaining barriers to realizing the full impact of CFE systems on biomanufacturing and applications of biotechnology.

Keywords

Automation; biomanufacturing; characterization; CFE; cell-free expression systems; TX-TL; scale-up.

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Summary

Cell-free expression (CFE) systems present a promising platform for new applications of biotechnology. Much of this potential remains unrealized, however, due to barriers to adoption, an unmet need for measurements and standards, and uncertainty as to how to leverage CFE within the larger context of cell-based manufacturing investments, among other challenges. Issues concerning scale-up and scale-out of CFE reaction volumes, along with effective and judicious use of automation, have emerged as consensus priority topics to advance the utility and impact of CFE in both academic and industrial settings. Foundational to these topics is the unmet need for robust characterization of CFE systems, through shared protocols to prepare and store cell extracts, optimized expression protocols, reagents and analytical methods to quantify expression, and improved understanding, prediction, and reproducibility of CFE. Automation of some aspects of extract production, including cell lysis, addition of reagents, screening expression conditions, and product isolation would enable more robust CFE systems.

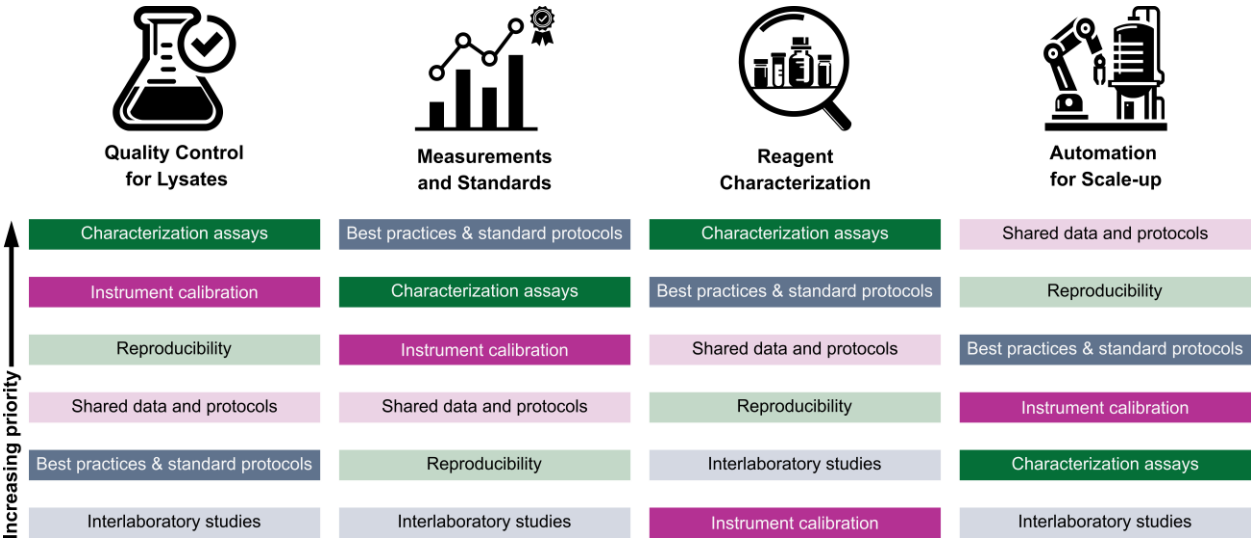

Fig. 1. Priority order of areas needed to advance CFE systems. Workshop participants joined four working groups focused on quality control for lysates, measurements and standards, reagent characterization, and automation for scale-up. Recurring topics emerged as priority areas needed to advance CFE systems and are ranked here based on discussions within each working group and the workshop as a whole.

To address this need, the National Institute of Standards and Technology (NIST) and the National Center for Advancing Translational Sciences (NCATS) convened the workshop Advancing Cell-free Manufacturing: Challenges in Scale-up and Automation in Rockville, Maryland in February 2024. This workshop brought together over fifty participants, representing the interests and needs of stakeholders in academic, industrial, and government settings. Together, through various plenary discussions, case studies, and working groups, participants broadly surveyed the field and focused on identifying both near-term and long-term needs to support CFE systems. This report synthesizes the workshop discussion and

presents actionable recommendations aimed at removing the remaining barriers to realizing the full impact of CFE systems on biomanufacturing and applications of biotechnology.

1. Introduction

Cell-free expression (CFE) systems are poised to revolutionize biomanufacturing and applications of biotechnology. Briefly, these systems[1, 2] harness the transcription and translation machinery native to cells, for example, RNA polymerases and ribosomes, respectively, for manufacturing products, such as proteins and small molecules, through the expression of natural or synthetic DNA. The characteristics, capabilities, and numerous advantages of CFE systems have been reviewed extensively elsewhere[3-6]. Some examples include: the open environment of CFE systems, allowing the ability to readily tune the composition of CFE reactions; decoupling growth and homeostasis from production, enabling the production of compounds that would otherwise be toxic to a living cell; portability for sensing applications or on-demand, distributed manufacturing; and, more rapid workflows and protein synthesis than cell-based workflows. Taken together, these and other capabilities position CFE systems to impact manufacturing significantly today and into the future. CFE systems have already been used to develop several existing and emerging products, such as pesticides, therapeutics, vaccines, and biosensors for environmental monitoring.

The most common CFE systems today across academic and industry settings are made from lysed cells–typically *E. coli* cells. In these systems, the cytoplasmic lysate is purified to remove endogenous genetic material and cellular debris. The resulting lysate is supplemented with additional reagents to provide the ribonucleotides, amino acids, an energy regeneration system to sustain translation, and biochemical cofactors to enhance expression of the desired end product. These systems are often prepared in-house but can also be purchased from an increasing number of commercial vendors.

CFE systems may also be reconstituted from isolated and purified cellular components, again most commonly from *E. coli* cells. These reconstituted systems include transcription and translation machinery, and associated recombinant proteins, while excluding tRNAs. Such CFE systems lend themselves to studies advancing the fundamental understanding of CFE reactions and related phenomena and systems, and often find use in academic settings. Due to their nominally known composition and analytically tractable number of components, reconstituted CFE systems are preferred for computational modeling of the biochemical properties of transcription and translation. These systems may be purchased from commercial vendors or, increasingly, prepared in-house.

Scale-up of biological systems from benchtop or discovery phase (μL to mL), to pilot phase (mL to L), and to commercial phase (L and beyond) remains nontrivial. There are few published success stories of CFE reactions scaled up from volumes of microliters to liters[7-9]. Common techniques include the use of bespoke protocols that, for example, rely heavily on the available laboratory equipment, specific type and composition of the CFE system, and environmental conditions of the laboratory, and may not scale successfully beyond the discovery phase. Optimizing protocols and processes beyond the benchtop scale would benefit from predictive knowledge and the development of measurements to inform which processes are technically and economically feasible to scale up to the pilot phase.

Box 1: Definitions and Acronyms

AI: Artificial intelligence.

Biological replicate measurement: One of multiple measurements intended to resolve variability in measurement results attributable to relevant biological processes[10].

Cell-free biomanufacturing: The use of cell-free expression systems to produce proteins, chemicals, small molecules, and other products.

CFE: Cell-free expression.

Comparability: Suitability of quantitative comparison between measurements obtained under different conditions[10].

DoE: Design of experiments; a systematic, rigorous approach to designing experiments that leverages statistical approaches to determine the relationship between experimental factors and the measured output.

Extract: Lysate that has undergone further processing, for example, with a subsequent runoff reaction or dialysis.

Lysate: Result of a liquid culture of cells disrupted, for example, through sonication, and clarified to remove cellular debris, endogenous DNA, and RNA.

Reproducibility: Measurement precision under conditions of measurement that include different locations, operators, measuring systems, and on the same or similar objects and protocols[10].

Scale-up: Steps involved in progressing a biomanufacturing process or biological system from benchtop or discovery phase (μL to mL), to pilot phase (mL to L), and to commercial phase (L and beyond).

Scale-out: A mode of increasing manufacturing capacity by using multiple bioreactor vessels of smaller volume working in parallel or in different locations.

Technical replicate measurement: One of multiple measurements intended to resolve variability in measurement results attributable to the measuring system, objects, or protocol[10].

TXTL: Transcription-translation.

Laboratory automation can facilitate more reproducible and effective CFE systems, by reducing variability in performance due to uncontrolled laboratory conditions and protocol steps, for example, due to human errors in pipetting, and by generating large data sets that can be used for process optimization through modeling and AI approaches. Automation has successfully increased the throughput of benchtop protocols and improved the reproducibility of CFE reactions[11, 12]. While the availability of automation tools and cloud laboratories is

expanding, the use of these tools often remains cost prohibitive to academic laboratories and startups, with few published protocols to provide a starting point for transitioning a benchtop protocol to an automated workflow.

Although stakeholder engagement informed the title and initial emphasis of the workshop on scale-up and automation for CFE systems, discussion during the workshop quickly indicated that foundational measurements and standards required focused attention first. A recurring theme throughout the discussion called for improving CFE systems, workflows, and measurements at a research scale prior to scale-up.

With investment and attention paid to the topics and recommendations presented in this report, the CFE community can rightly look ahead to a horizon of impressive and far-reaching impact for this technology.

2. Recommendations

This section presents actionable recommendations to advance the field of cell-free manufacturing distilled from discussions held at the workshop.

Identify metrics and develop assays to characterize CFE system composition and performance.

- Define metrics that are informative and fit-for-purpose for prediction, design, and scale-up.
- Develop assays for the amount and activity of the components in CFE systems.
- Establish tools and methods to assess and decouple transcription and translation in CFE reactions for independent measurements and quality controls.
- Develop and disseminate characterization assays in formats suitable for both manual and automated workflows with accompanying protocols and documentary best practices.
- Apply a multiomics approach for comprehensive understanding of CFE systems at many time points.

Develop protocols, best practices, and reference materials for CFE systems.

- Establish community-accepted best practices and shared protocols for preparing and characterizing lysates, reagents, and assembled CFE reactions.
- Develop and distribute reference materials, such as a defined lysate, reference DNA template, and reference fluorescent reporter.
- Improve sourcing and characterization of materials with appropriate purity and grade or develop a separate grade of chemicals suitable for preparing CFE reagents.
- Integrate measurement calibration into CFE workflows to improve comparability and reproducibility.
- Validate quantitative assays to demonstrate measurements are fit-for-purpose.

Improve reproducibility for CFE systems.

- Leverage characterization assays and laboratory automation to identify sources of variability.
- Establish a minimal information standard for transparent reporting of measurements, methods, and results.
- Incentivize routine reproducibility studies spanning different CFE systems and applications.

Leverage laboratory automation for CFE workflows to facilitate scale-up.

- Develop and share automation methods compatible with common automation tools and hardware.

- Integrate sensors for real-time, in-line, and continuous measurements into automated workflows for relevant measurands.
- Develop commercial kits and reagents specific to CFE systems, for both manual and automated workflows, to reduce the burden of scale-up.
- Build laboratory hardware specific to CFE systems at different scales.
- Apply statistical approaches and partner with statisticians to take full advantage of large datasets.
- Leverage emerging AI tools and large datasets to support and optimize scale-up.

Build infrastructure and knowledge to enable successful scale-up of CFE systems.

- Expand CFE workflows to a broader variety of organisms, beyond *E. coli* and engineered or minimal cells.
- Apply approaches from synthetic biology to engineer cells to optimize CFE reactions at scale.
- Gain predictive understanding to inform the selection of CFE systems and effective workflows for scale-up.
- Apply sustainability assessments, such as life cycle assessment (LCA), and risk assessments, such as technoeconomic analysis (TEA), during scale-up.

Support a vibrant community for discussion and the sharing of resources between users of CFE systems.

- Develop a common lexicon to improve communication within the CFE community and with commercial vendors, regulators, policy makers, and the general public.
- Share protocols, best practices, and data, including negative results, to accelerate technology transfer and speed innovation.
- Organize and participate in interlaboratory studies to assess materials and methods for the preparation and assembly of CFE systems, towards improved reproducibility and wider adoption.
- Build a collective knowledge base through the establishment of a consortium or regular meetings to address common challenges.

3. Working Groups

The workshop discussion was structured in part around working groups. There were five working groups, with participants free to choose which discussions to engage, on the topics below. The topics for the working groups were based on outreach and engagement with the CFE community prior to the workshop. The Data working group was dissolved due to a lack of interest, and issues of data collection, storage, access, and sharing are included, as appropriate, in the discussions of the other working groups below. This reflects the current priority placed on other areas over data challenges for the CFE community.

3.1. Quality Control for Lysate Preparation and Characterization

This working group convened to identify methods and measurements to benchmark the lysates that comprise a key component of a CFE reaction. Participants agreed that reproducibility of lysate composition and performance remains an outstanding challenge, and quantitative measurements of lysate productivity would help ensure lysates are fit-for-purpose.

E. coli continues to form the basis of most lysates used for CFE in both academic and industrial settings. This choice builds upon legacy protocols and workflows in many laboratories, reflecting the long history of *E. coli* as a model organism in cell biology. Briefly, cell lysate is prepared by disrupting cells grown in liquid culture to exponential phase, for example, using sonication. After disruption, the crude lysate is clarified to remove cellular debris and endogenous DNA and RNA. The resulting solution contains transcription and translation machinery needed for protein synthesis. The cell lysate can be further processed into an extract, for example, with a runoff reaction to allow endogenous transcription and translation to come to completion and enable further degradation of native genomic material[13] or with dialysis to remove molecular components smaller than 10 kDa[1]. In this report, measurements and characterization for lysates are assumed to apply also to extracts.

Currently, reproducibility of the lysate preparation is often confounded with reproducibility of the entire CFE reaction; separate consideration of the lysate itself would illuminate the impact of attributes specific to the lysate, for example, differences in the composition of the lysate, differences in preparation methods, and variability between batches or lots. Lysates largely remain black boxes, and fundamental understanding of reproducibility and performance is lacking. To compensate for the quality of the cell lysate, a single researcher often prepares lysates for an entire laboratory to ensure that lysates are productive, perform as expected, and are adequately reproducible for the needs of that laboratory. With regard to measurements, it is common for the entire CFE workflow to reach completion before an end-point measurement is taken and may indicate that the lysate behaved poorly. Specifically, the performance of the lysate is often assayed by measuring the amount of protein expressed by the CFE system, which includes the lysate, the DNA template, and any supplementing reagents necessary for protein synthesis. These assays measure the translation of a fluorescent reporter in the entire CFE system, which convolves effects of every component of the CFE reaction and may not indicate the performance of the lysate itself.

Quality control measurements of lysates–independent of measurements of the protein expressed by the assembled CFE reaction–are not routine and typically consist of a limited number of endpoint spectroscopic assays, such as Bradford and bicinchoninic acid (BCA), to measure total endogenous protein concentration in the lysate. For lysates from *E. coli*, a protein concentration of (30 to 50) mg/mL[1] is considered one informal indicator of a productive lysate. Additionally, quantitative comparison of spectroscopic measurements across tools and laboratories requires appropriate calibration of laboratory equipment, such as plate readers, which is often not done. Consequently, spectroscopic measurements cannot be compared across laboratories, for example, due to variations in plate reader equipment.

The development of fit-for-purpose quality control measurements for lysates is hindered, in part, by the numerous types of CFE systems currently in use and the broad range of applications these systems support. For example, lysates for diagnostic and screening applications generally require rapid response times, while lysates for biomanufacturing applications must be productive over longer timescales to increase product yield. No community consensus exists on which attributes of a lysate should be assayed—such as proteins, endogenous DNA, or production rate—to benchmark whether a lysate is fit-for-purpose. Additionally, quality control and characterization of lysates may differ across scales, from discovery phase to commercial phase. For example, discovery workflows may focus on screening for optimization, which can include measuring lysates from different organisms and strains, to rapidly identify the lysate(s) most appropriate for the target application. During pilot and commercial phases, measurements of batch-to-batch variability and productivity across scales may emerge as significant, for example, to ensure sufficient reproducibility for biological licensure applications. To meet regulatory compliance for a diagnostic product or manufacturing process, quantitative and comprehensive characterization of the lysate may be of particular importance.

To identify differences between “good” or “bad” lysates, workshop participants called first for identifying measurands indicative of lysate performance. For the purpose of this report and because lysate performance is application specific, good lysates are defined as productive and fit-for-purpose. Similarly, bad lysates are defined as inactive and not fit-for-purpose. While general attributes of good lysates may be shared across different CFE systems, specific performance criteria of a lysate should be agreed upon prior to each study or use case. Various measurements for benchmarking lysate performance were discussed in this working group, which should be selected as appropriate to each target application or scale. First, identifying critical parameters during cell growth, such as the bacterial conditions for inoculation of the growth vessel, growth kinetics, time of harvest, growth phase of harvest, nutrient consumption, and oxygen profile, could inform how the state of the host cells contributes to lysate performance. For example, lysates prepared with cells collected in exponential phase are often considered more productive than lysates prepared with cells collected in stationary phase, in terms of the amount of protein expressed[14]. Second, a multiomic approach that includes, for example, a proteomic analysis using high performance liquid chromatography with mass spectrometry (LC-MS) coupled with a transcriptomic analysis using next generation sequencing, could offer in-depth characterization of lysates from different host organisms, strains, across batches, and across scales. This has the potential to generate large data sets and identify

relationships between lysate composition, preparation methods, and performance not previously known. Unfortunately, the cost of these quantitative assays can be prohibitive due to lack of instrumentation, funding, or expertise. Furthermore, no framework exists for comprehensive analysis of this type of data from lysates, which remains a significant challenge. Third, enzyme-linked immunosorbent assays (ELISA) could be used to identify the presence or absence of proteins, such as ribosomes and elongation factors, in the lysate. Fourth, functional assays could further investigate the bioenergetic and expressional activity of lysate components directly involved in translation, for example, ribosomes, and other processes, such as glycolytic intermediates correlated with lysate productivity. Fifth, the translation potential of the lysate could be assayed by titrating increasing concentrations of an antibiotic, such as chloramphenicol, to determine the concentration of antibiotic that causes a two-fold decrease in the production of a fluorescent reporter. This concentration, analogous to a half-maximum, could be used to compare the translation potential of different lysates, with a lower value of the half-maximum denoting lower translation potential. For accessing transcription potential, for example, of *E. coli* RNA polymerase, a similar titration could be performed with rifampicin. Sixth, gel electrophoresis of ribosomal RNA (rRNA) could be used to benchmark rRNA maturation towards a better understanding of ribosomal biogenesis and activity in the lysate. Other measurements, beyond those listed here, could also be relevant.

Researchers in academia, industry, and government can each play important roles towards making productive lysates that are fit-for-purpose. These various users of CFE systems have different goals for this technology, which is reflected in the types of measurements and requirements for the performance of these systems. Academic researchers can support good lab practices for developing and testing protocols for lysate preparation, as well as facilitate knowledge transfer by publishing scientific studies with detailed protocols for lysate preparation and characterization. Industry could collaborate constructively with academic researchers, for example, by providing lysates for testing variation in composition and preparation methods and across batches and scales. Government entities can lead or participate in interlaboratory studies to assess the effects of materials and methods used during lysate preparation on lysate productivity, towards establishing which parameters significantly affect lysate performance and their mechanisms. Additional interlaboratory studies could assess the performance of different lysates for biomanufacturing various products that span the range of applications for CFE systems. National metrological institutes, such as NIST, along with standards-setting organizations, can develop reference materials or other tools for measurement assurance for a fluorescence reporter specific to CFE systems to benchmark lysate performance, as well as documentary standards for lysate production and benchmarking, including both measurements of transcription and translation. Development of a reference lysate could enable academic researchers, government scientists, and industry to benchmark the composition and performance of their lysates, facilitating comparability and validation of the preparation and performance of CFE systems. Similarly, the development of reference DNA and RNA templates could facilitate comparability of the transcription and translation potentials of CFE systems, respectively. These or other entities could also provide independent validation and verification or testing and evaluation for studies of lysate composition and preparation to increase reproducibility.

3.2. Analytical Measurements and Standards

This working group convened to discuss characterization and benchmarking of the performance of CFE systems across scales, from discovery phase (μL to mL), to pilot phase (mL to L), to commercial phase (L and beyond), towards improved reproducibility and wider adoption. Measurements are foundational to reproducibility, comparability, optimization, and successful technology transfer across scales and biomanufacturing sites. Working group participants agreed on the need for quantitative and fit-for-purpose measurements and standards to enable reproducible CFE systems across scales and applications. Participants discussed the need for common definitions to facilitate communication amongst the technical community and with external stakeholders, key metrics for measuring the performance of CFE reactions, calibrants and methods to ensure that measurements are quantitative and comparable across laboratories and scales, and sharing of data, including data from positive and negative controls, to facilitate a common understanding of how CFE data should be interpreted. These topics remain outstanding challenges to developing fit-for-purpose measurements and standards for CFE systems.

Because CFE systems are commonly used to express proteins, such as the fluorescent reporter described below in Box 2, measurements for translation offer one approach to quantifying the productivity of a CFE reaction at any scale. Workshop participants proposed an assay for measuring translation in terms of the specific activity of a protein expressed by the CFE reaction. This approach depends on the incorporation of unnatural amino acids into the protein being expressed, for example, at the C-terminus. The unnatural amino acid could either emit fluorescence or bind covalently to a fluorescent reporter[15]. Workshop participants also discussed the potential for expanding the use of 2-dimensional gel electrophoresis for qualitative characterization of translation, such as using SDS-PAGE to separate proteins by mass and native-PAGE to separate proteins by protein complex mass. Because these techniques are well-established and the resulting gels have a well-defined and consistent background signal for imaging, automated image processing with AI tools could increase the throughput and sensitivity of these techniques[16].

Beyond translation, characterization of CFE reactions will require measurements for benchmarking the “process health” of the CFE workflow across scales and applications. For example, this could include measurements of yield, quality, throughput, efficiency, and waste products, as well as life cycle assessment (LCA) and technoeconomic analysis (TEA), to identify, for example, sources of variability and parameters for workflow optimization. Unfortunately, the community lacks sufficient knowledge of which specific process health indicators are significant for CFE workflows, which has stymied discussion on which metrics should be prioritized. Workshop participants called for the development of a multi-omics approach to benchmarking CFE reactions as the first step to identifying metrics for transcription and translation to inform process health across scales. These measurements could later form part of a suite of assays for real-time monitoring of process health at each scale.

Workshop participants discussed the need for increased reproducibility in CFE reactions. Specifically, a better understanding of the sources of variability in CFE would elucidate the appropriate type and number of replicates that should be measured for a specific CFE system or

application. For example, biological replicate measurements are intended to resolve variability in measurement results attributable to relevant biological processes[10]. To date, few published studies have explored the effects of biological variability on CFE performance, and few workflows measure more than one biological replicate. Technical replicate measurements are intended to resolve variability in measurement results attributable to the measuring system, object, or protocol[10]. Anecdotally, most studies measure up to three technical replicates per experiment, with as few as one technical replicate in rapid screening experiments. One approach to better understand sources of variability in CFE systems is to partner with statisticians and experts in experimental design to determine the appropriate type and number of replicates necessary for a specific measurement or workflow and sufficient to yield the desired confidence in the measurement.

The use of standards, reference materials, and instrument calibration is not routine, which hinders reproducibility, comparability, and technology transfer. CFE reactions are commonly measured using a multimode plate reader to observe the fluorescence from a reporter molecule, such as a protein or aptamer, in arbitrary fluorescence units (AFU). There are several different types of calibration that can be performed to ensure that plate reader measurements are comparable across users, laboratories, and manufacturers. For example, a manufacturer's calibrations can verify that the linearity of signal, limits of detection, and alignment of the optics are within manufacturer’s specifications. Beyond a manufacturer’s calibration, a plate filled with a solution with a uniform fluorescence can check for variation in the detected signal across the plate, and an empty plate can test the background signal from the plate itself. A calibration curve can also be used to convert measurements of arbitrary units to absolute units to facilitate quantitative comparison between measurements[17]. For example, if the CFE reaction expresses an enhanced green fluorescent protein (eGFP), a calibration curve consisting of a dilution series of purified eGFP prepared in the CFE system without a DNA template would facilitate the conversion from AFU to absolute units of concentration, such as micrograms per milliliter. Some purified fluorescence reporters are available commercially[18] but are typically prepared in buffers that may adversely affect the CFE reaction, for example, by altering the salt balance. Anecdotally, the reported concentration of the commercial, purified fluorescence reporters varies between lots–and sometimes between aliquots from the same lot–and therefore should always be verified in the laboratory in terms or protein concentration and purity. Fluorescent reporters that are not available commercially must be prepared in-house in sufficient quantities to ensure that a single lot can facilitate comparison between instruments, users, and laboratories. Workshop participants discussed the need for commercially available reference materials for a variety of common fluorescent reporters, with suitable purity, stock concentration, and solvent for use with CFE systems. Participants also called for guidelines and best practices for instrument calibration to facilitate comparability for measurements across sites, researchers, and instruments.

While the expression of fluorescent reporter proteins may be appropriate for positive control experiments, workshop participants expressed a general desire to move beyond measurements of fluorescent reporters for quantifying the productivity of a CFE reaction. A panel of more structurally complex proteins of different classes could be used to determine the productivity of a CFE reaction in terms of transcription and translation. While both time and labor intensive,

such a panel could be used to screen CFE workflows, for example, when identifying the potential and cost of scale-up or considering new applications of a particular CFE system.

Box 2: A common CFE workflow: expression of eGFP

A common workflow for users of CFE systems is the expression of a fluorescent reporter, often enhanced green fluorescent protein (eGFP) or its derivative deGFP, in an *E. coli* CFE system. CFE systems based on *E. coli* are commercially-available platforms accessible to many researchers, due to the large number of technical publications and protocols available across economic sectors and for a variety of applications. These commercial CFE systems can be lysate-based[19] or reconstituted[19-21]. eGFP is a common fluorescent reporter expressed in these systems, with a short maturation time of (5 to 10) min. This fluorescent reporter is also commercially available as a recombinant stock solution at known concentration for use in the construction of a calibration curve to quantify the amount of eGFP produced in absolute units by the CFE reaction. This common workflow offers a positive control experiment to test the performance of a CFE reaction by observing the amount of protein produced, typically as an endpoint measurement. Fluorescence emitted by the fluorescent reporter protein is often measured using a plate reader and presented as the fluorescence intensity in arbitrary units as a function of time. Infrequently, a concentration is reported in units of mass per volume, for example milligrams per milliliter, of protein produced over the duration of the reaction. This common, endpoint measurement has become a *de facto* "standard" in the CFE community.

The expression of a fluorescent reporter protein, like eGFP, also provides a useful, straightforward workflow to consider analytical measurements and standards for protein production using CFE systems, as well as best-practices for protocols and data sharing. However, production of the various common versions of GFP may not provide a sensitive assay of CFE performance, because these fluorescent proteins have been engineered for robust expression and folding. While they can offer a rapid, easy assessment of whether a CFE reaction produces any protein at all, the fluorescent reporter may differ significantly from a target protein of interest, which may be more complex than GFP and not share these robust qualities. This suggests the additional need for more sensitive reporter proteins than GFP to better mimic proteins of interest to the CFE community.

A shared understanding of how to interpret data from CFE systems would speed the development of measurements and standards towards improved reproducibility and address challenges in interpreting and comparing existing and future data, including data from failed experiments. Workshop participants agreed that time-course measurements of the product

expressed in the CFE reaction should be favored over endpoint measurements, despite the time, labor, and costs involved to obtain a more complete and informative view of a CFE reaction. Whenever possible, measurements should be reported as a reduced quantity, such as a mean value, with uncertainty (Figure 2A) and include a baseline from negative control measurements. Several recent studies have commented on metrics available from a time-course measurement of protein expression in a CFE workflow, for example, for the purpose of reaction optimization[22] and the development of predictive modeling tools[23]. Workshop participants emphasized several of these metrics, displayed below in Figure 2:

- Maximum yield of product expressed;
- Time to reach the maximum yield of product expressed, as the time from the start of the measurement to the time to reach the maximum yield;
- Maximum rate of product expression, as the maximum linear rate of production;
- Time to reach maximum rate, as the time from the start of the measurement to the time to reach the maximum rate of expression;
- Inflection time, as the time from the start of the measurement to the time at which the rate of product expression begins to decrease;
- Percent decline, as a decrease in the amount of product expressed from the maximum yield; and,
- Time to percent decline, as the amount of time from the start of the measurement to the time to reach a predefined decrease in the yield of product expressed after having reached its maximum yield of product expressed.

Another potential metric, not shown in Figure 2, is time to reach half-maximum, as the time from the start of the measurement to the time to reach one half of the maximum yield of product expressed. Characterizing time-course data in this way may also illuminate the causes of common but poorly understood artifacts in data from both CFE reactions (Figure 2B) and negative control reactions (Figure 2C).

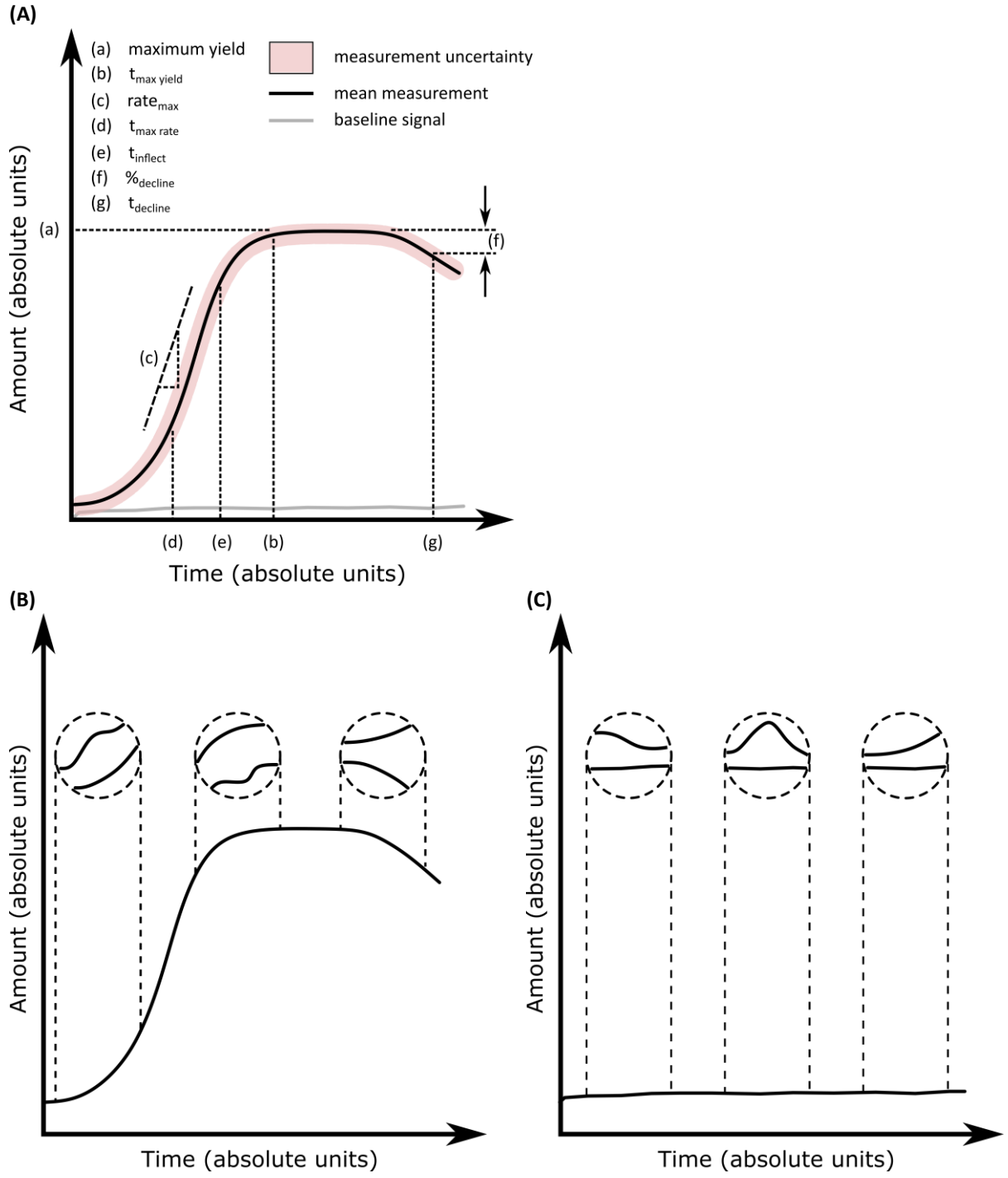

Fig. 2. Representative schematic of time-course data collected during protein expression in a CFE reaction, showing candidate metrics for reaction characterization. (A) The black trace corresponds to the mean of the measurement and the shaded area indicates the measurement uncertainty. Suggested metrics include: (a) maximum yield; (b) time to maximum yield; (c) maximum rate; (d) time to reach maximum rate; (e) inflection time; (f) percent decline; and, (g) time to percent decline. Time-course measurements of CFE reactions **(B)** and negative control reactions **(C)** can include common but poorly-understood artifacts.

A minimal information standard for reporting on experiments using CFE systems, analogous to previous efforts in bacterial cell growth[24] and recombinant proteins[25], could offer a near-term deliverable to support transparent data reporting and help identify which experimental factors contribute significantly to variability and productivity. Examples of candidate metrics include but are not limited to:

- Liquid cell culture growth parameters, such as media type and inoculum age;
- Cell harvest parameters, such as centrifugation speed and duration;
- Cell lysis parameters, such as disruption method;
- Post lysis parameters, such as type of dialysis cassette;
- Reagent assembly parameters, such as composition, purity and grade of each component, and order of assembly; and,
- CFE reaction parameters, such as volume and container type.

Informed by a suite of characterization assays, this standard for reporting would generate datasets to help identify approaches to improve CFE reproducibility, productivity, and scalability, and lead to better predictive modeling tools to identify sources of variability not previously known.

3.3. Reagent Characterization

This working group convened to discuss the characterization of reagents to supplement both lysate-based and reconstituted CFE systems, by providing the necessary energy, cofactors, salts, substrates, nucleotides, and amino acids for transcription and translation. CFE systems are sensitive to contamination and the quality of reagents supplementing these systems. Workshop participants agreed that quality control for reagents remains an outstanding challenge, and consensus on best practices for reagent preparation and characterization would help ensure reagents are fit-for-purpose. Workshop participants also called for the development of commercial reagents and kits specifically for CFE systems, as well as a separate grade of chemicals suitable for the preparation of reagents for CFE systems. While the composition of reagents may differ depending on the specific CFE system being supplemented, one common challenge with preparing these reagents stems from sourcing the necessary components.

With regard to sourcing reagents for CFE, tRNAs and small molecules, such as folinic acid, magnesium, potassium, ammonium glutamate, nucleoside triphosphates (NTPs), nicotinamide adenine dinucleotide (NAD), coenzyme A (CoA), oxalic acid, putrescine, spermidine, and phosphoenolpyruvate (PEP), can be purchased commercially as stock solutions or as powders, the latter of which can be resuspended in an appropriate solvent to reach the desired concentration. If there is no detriment to their function, these tRNAs and small molecules may be combined into a single stock solution as a “master mix” to facilitate rapid reaction assembly. Anecdotally, workshop participants noted that purchasing small molecules of the highest purity available, for example, molecular biology or HPLC grade with greater than 99% purity, and from a trusted vendor supported a high quality and reproducible master mix. Also essential for the

expression of proteins is the preparation of a stock solution of amino acids, typically composed of all twenty amino acids in powder form and resuspended in water. Previously, amino acids could be purchased premixed in powder form; because this product has been discontinued, each amino acid must now be purchased individually.

Natural or synthetic DNA templates can be obtained from DNA synthesis companies or repositories, such as Addgene[1][26], Twist[27], or IDT[28]. Plasmid DNA is extracted from cells, which can be performed using a variety of commercial DNA extraction kits that vary in extraction methodology, adsorption methodology, elution methodology, duration, and yield. Certain components of these kits can be repurposed to purify linear DNA; alternatively, kits developed specifically for processing linear templates and PCR products can be used. Templates can then be further modified in the laboratory through common methods, such as Gibson and Golden Gate assembly. Additional purification of any DNA template is strongly recommended, to ensure that the solution containing the DNA template is free of contaminants at concentrations that could inhibit transcription or translation. This type of purification may include multiple washes and precipitation steps and is generally performed using commercial DNA extraction or PCR purification kits.

Reagent sourcing is further complicated by availability across geographic regions; for example, not all products are available in every country, some products are often out of stock due to high demand, and the choice of vendor may be limited as a result of mergers and acquisitions across manufacturers. Overall, the CFE community is aware of and acknowledges the potential effect of using reagents from different sources and generally discloses in the technical literature the manufacturers of reagents used in a study. However, no published studies to date have considered systematically the effects of the source of reagents on the CFE reaction. This type of effort could identify the parameters, such as purity and grade, that would ensure reagent components are sourced appropriately for CFE applications.

Another common challenge for reagent preparation stems from disagreement between protocols used in the CFE community to prepare nominally identical reagents. The stock solution of amino acids to provide CFE systems with the essential building blocks of proteins is particularly difficult to prepare[29]. Because several amino acids, such as phenylalanine and tyrosine, have very low solubility in water, and others, such as leucine and isoleucine, are hydrophobic, preparation of this stock solution is not straightforward and often results in a mixture with precipitate. Tricks for increasing the solubility of amino acids through the use of heat, agitation, or other means differ across laboratories and are not well documented in scientific publications. Additionally, published protocols differ in the order in which individual amino acids are added when preparing the stock solution and in the recommended final concentration of each amino acid in the stock solution[14, 30].

Workshop participants also noted the need for reagent characterization and quality control across lots, which is not performed routinely due to constraints on resources and time. Premixing reagents into concentrated stock solutions or master mixes facilitates rapid assembly

[1] Certain equipment, instruments, software, or materials, commercial or non-commercial, are identified in this paper in order to specify the experimental procedure adequately. Such identification does not imply recommendation or endorsement of any product or service by NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

of CFE reactions by reducing the number of individually added components, but this practice can confound quality control. Measurements of stock solutions may not identify which specific component of the master mix accounts for observed difference in quality or performance. For example, tRNAs and small molecules are rarely characterized independently prior to combination into a single stock solution. With respect to DNA templates, most laboratories have tools in place for spectrophotometric measurements to characterize DNA concentration and assess quality in terms of purity as determined by contaminants in the solution containing the DNA template; however, these measurements may not be performed routinely. Measurements of absorbance to determine DNA concentration are typically favored over more labor-intensive but more accurate fluorometric measurements, despite known discrepancies between the two methods[31]. One recent study explored the effects of DNA template preparation, including concentration of the DNA template, quality of the DNA template in terms of physical damage, and the quality of the DNA solution in terms of purity, on protein expression in a lysate CFE system[32]. Few similar efforts exist to explore other reagents and their isolated effects on CFE reactions. Such studies are often prohibitive, due to requirements for specialized techniques not widely accessible that require significant investment in training and infrastructure. For example, measurements using high performance liquid chromatography (HPLC) can benchmark the concentration of individual amino acids in the amino acid stock solution. Opportunities also exist for using established quality control methods from industrial settings, such as chemical manufacturing workflows, to inform the type of characterization and quality control that could be developed for reagents supplementing CFE reactions.

Compared to lysate-based systems, the characterization of reconstituted CFE systems, such as PURE[1], requires additional considerations. Reconstituted CFE systems available commercially contain purified proteins[20, 21], which can be considered another type of reagent, present at a defined yet undisclosed formulation. Variation in performance across lots has been observed anecdotally but not investigated systematically. These purified proteins could be characterized individually to better understand how attributes, such as protein purity and activity, affect the performance of reconstituted CFE systems. For example, reconstituted systems lacking ribosomes may allow benchmarking of the quality of ribosomes isolated in the laboratory. Vendors of reconstituted systems could offer individual, purified proteins as spike-in reagents, to determine the optimal protein concentrations in both reconstituted and lysate-based systems. Additionally, interlaboratory studies could compare the composition and concentration of reconstituted proteins between commercial and laboratory-produced reconstituted CFE systems to identify best practices for the formulation of reconstituted systems. Beyond the individual proteins for reconstituted systems, these studies could also help to explain differences observed in productivity across reconstituted systems produced in different laboratories and from different protocols[33, 34].

To address challenges with reagent preparation and characterization, workshop participants called for more accessible sharing of protocols and data. The CFE community has recently published protocols and best practices[11, 35] to improve reproducibility and aid new users. One such publication for the preparation of circular DNA templates included four individual protocols for DNA extraction and purification (manual filter-based, manual magnetic bead-based, automated filter-based, and automated magnetic bead-based) using commercial kits

and reagents. This publication included advice to improve the kits' performance and resulting DNA yield, along with several methods for characterizing DNA concentration and quality in terms of contaminants in the DNA solution and amount of DNA breakage. The CFE community has also been part of the growing practice in the scientific community to deposit published experimental data in data repositories, such as Git and GenBank, with many academic journals requiring this for publication. These efforts to share protocols and data publicly should continue to grow. For example, protocols for preparing more complex reagents, such as amino acid stock solutions, could greatly reduce the challenge of preparing this particular reagent and identify metrics for characterization and quality control. Workshop participants called for results from failed experiments to also be widely available, for example, reagent formulations that were not successful, fit-for-purpose, or detrimental to CFE reactions.

A comprehensive examination of reagents is required to address characterization needs for the broad range in type and composition of reagents for different CFE systems. A thorough literature review can provide a preliminary list of reagents common across most CFE systems, including information on the manufacturer, part number, grade, and concentration. This can inform a suite of characterization assays for benchmarking the effects of composition and quality of reagents on CFE, perhaps in a multiwell format. Interlaboratory studies that leverage these characterization assays would provide valuable data on reagent composition for many CFE systems and can be shared with the community through peer-reviewed publications. The resulting data could spur commercial development of fit-for-purpose reagents and speed the development of predictive models to inform reagent optimization for particular CFE systems and applications. This would enable the community to better consider standards regarding reagent preparation and characterization, for different use cases and end applications.

Equipped with a clearer understanding of how the composition and quality of reagents affect CFE reactions, the CFE community can build infrastructure to improve reproducibility and reduce the burden of scale-up through community-accepted standards and measurement assurance. Workshop participants called for identifying the minimum set of criteria to include in specification sheets provided with the purchase of reagents. For example, purity, grade, sterility, and composition, as well as other relevant practices or standards used, such as cGMP practices. Formulating specification sheets that are machine readable will ensure that automated laboratory workflows can track changes to reagent sourcing and composition. Partnering with commercial chemical suppliers and CROs can lead to the formation of a separate grade of reagents suitable for CFE. This could reduce dependence on existing grades of chemicals that fluctuate in availability depending on supply and demand and provide more resilience to CFE biomanufacturing at scale. Through the establishment of private-public partnerships, such as a consortium, the CFE community could further democratize the sharing of protocols and data. Academic researchers can develop and test protocols for reagent preparation and characterization, as well as facilitate knowledge transfer by publishing scientific studies with detailed protocols. Industry could collaborate constructively with academic researchers, for example, by providing reagents for testing the variation in composition and preparation methods across batches and scales. Government entities can lead or participate in interlaboratory studies to assess the effects on reproducibility of materials and methods used during reagent preparation. Finally, by exploring best practices for commercial

biomanufacturing, for example of mRNA vaccines, the community can identify which existing standards and metrics from adjacent industries can be modified to support the production and characterization of reagents at scale for a particular CFE system or application.

3.4. Role of Automation and Scale-up

This working group focused on tools and methods for scale-up and scale-out, including distributed manufacturing, of CFE systems. Workshop participants noted the breadth of relevant scales, from the use of laboratory automation for parallel CFE reactions during research and discovery at nL to μL volumes, to pilot studies in benchtop bioreactors at mL or L volumes, and beyond for commercial production using industrial fermenters. Scale-out in the form of distributed manufacturing was discussed only briefly and in the context of rapidly deploying smaller, more agile manufacturing facilities than traditional facilities and workflows. Scale-out has the potential to reduce operational risk, increase flexibility in manufacturing capacity, and increase paths to process validation. Examples of the need for this capability include worldwide, on-demand production of therapeutics during disease outbreaks or defense applications at competitive cost and availability. Other types of scale-out for CFE systems, to expand the types of organisms used to make lysate and portable sensors based on CFE, were discussed briefly in the other working groups.

The role of laboratory automation[36, 37] in engineering biology research and discovery is becoming increasingly commonplace. This trend is reflected, for example, in the establishment of the Global Biofoundry Alliance[1][38, 39] and cloud laboratories[40-42]. Laboratory automation can help to identify the relevant process parameters to monitor and tune for each workflow or application of CFE, potentially improve reproducibility by reducing human error, and generate large amounts of data, including from failed experiments, for modeling and for prediction of bioprocess development. Higher throughput, as compared to manual workflows, can also increase the amount of product expressed, the number of designs screened or optimized, and allow the use of statistical approaches, such as Bayesian optimization or design of experiments (DoE), to further optimize CFE workflows. During the discovery phase, scale-up relies on liquid handlers[17, 43-45] or microfluidic chip technologies[46, 47] to assemble CFE reactions at nanoliter to microliter volumes. Benchtop culture systems enable high-throughput continuous cell growth experiments[48, 49] and could be used to optimize growth conditions for CFE across volumes of milliliters to liters, for example, toward more reproducible lysates or documentary standards for the preparation of reconstituted CFE systems. Some automated cell lysis systems, such as high throughput ultrasonic and high-pressure homogenizers, and cryo-pulverization systems for tissue processing are available commercially and could be adapted among academic and industrial practitioners to improve reproducibility in protocols. In addition, modular laboratory automation, such as those piloted by Zymergen[1] and Ginkgo Bioworks, can facilitate scale-out through increased flexibility of automated workflows and facilitate technology transfer between sites and researchers.

Workshop participants called for community-accepted guidance on requirements for scale-up and scale-out of CFE systems, which is currently lacking. This guidance would address both biological and technical challenges to be met by automation systems. For example, ribosomes

are sensitive to shear force, which restricts methods for reaction mixing and homogenization to those that limit shear stress[50]. In another example, the air-water interface may change when transitioning between scales, potentially affecting formation of air bubbles and foaming, which may degrade some proteins by promoting structural degradation and aggregation[51]. While workshop participants did not provide specific details, they described scaling-up CFE systems for protein expression from micrograms to grams as relatively straightforward. Challenges in scale-up arise when going beyond grams to kilograms of protein expressed, in part due to cost, lysate upcycling, DNA recycling, limited commercial availability of reagents, such as amino acids, lack of tools and best practices for optimizing at this scale, and insufficient process optimization at pilot and commercial scales.

After access and training, successful and routine scale-up and scale-out of CFE manufacturing will rely heavily on advances in measurement tools and methods, such as robust sensors, as well as CFE systems with reproducible composition and performance. These measurement tools should be fit-for-purpose, facilitate benchmarking biological processes at different scales, and may differ significantly from measurements for scaling-up cell-based manufacturing. Additionally, it remains unclear which specific metrics and measurements should be made to determine how a CFE reaction will scale, in part because published data and protocols of CFE systems from academic research do not typically include scaling beyond microliter volumes.

In-line and off-line sensing and analytical tools exist[52] but should be applied properly to ensure the resulting measurements are fit-for-purpose for CFE systems across scales. Ideal sensors would provide real-time, in-line, and continuous measurements in an array format for multiple characteristics, such as nutrients and indicators of metabolic health. For example, when scaling CFE systems for biomanufacturing in quantities of grams and kilograms, these sensors should be adapted to the bioreactors employed at that scale. Some examples of how these individual parameters contribute to CFE performance are listed in Table 1. Sensor measurements should be robust and scale with advances in infrastructure and data collection.

Another approach to identify potential rules for scale-up of CFE systems could be to measure the performance of a CFE system proven at commercial scales as the reaction volume is decreased or scaled down. Instead of monitoring individual reaction parameters described above, macroscopic performance of the system could include measuring protein yield, rate of protein expression, functional activity, and stability. This approach could identify specific parameters, such as nutrients, pH, temperature, mixing rates, and others, essential to optimizing the reaction at different scales and hint at fundamental biological processes important to scale-up.

Once reproducible data sets have been generated using automated screening and DoE techniques, AI methods can be employed for modeling and predictions for scale-up, for example, by identifying which parameters of the CFE workflow are critical at each scale. One such approach focused on using an evolutionary algorithm and existing published data to inform future experiments [53]. Another more recent example used an active learning algorithm to determine the composition of reagents necessary for robust CFE from a non-model organism[54]. However, these studies were not extended to scale-up and scale-out applications or to multiple different organisms and strains.

In summary, this working group identified key areas where automation and scale-up can significantly benefit the development and production of CFE systems. First, there is a need for community-accepted guidance, fit-for-purpose measurement tools, and reproducible data sets to enhance the predictability and efficiency of scaling-up CFE systems. Second, robust sensor technologies that can be applied for screening at microtiter plate platforms and adapted to the larger scale CFE bioreactors should also be developed. Third, future directions should focus on integrating advanced automation technologies and leveraging AI methods for modeling, optimizing, and predicting CFE system performance at various scales.

Table 1. Some examples of the characteristics of CFE systems that may be informative to monitor and control for scale-up and scale-out.

Parameter	Description	Measurand
Amino acids	Amino acids are building blocks of proteins and essential for translation of proteins.	Concentration
ATP	ATP is the predominant source of energy for the synthesis of proteins and nucleic acids.	Concentration
Dissolved oxygen	Oxygenation in CFE has been shown to affect the amount or yield of protein expressed. Oxygen is required for ATP synthesis via oxidative phosphorylation, which accounts for a majority of ATP synthesis in CFE systems [55, 56].	Percent of oxygen dissolved of the maximum possible oxygen dissolved in solution
DNA template	DNA, whether circular or linear, is essential for transcription of RNA, and must be of sufficient quantity, quality, and integrity [32].	Concentration Contaminants Amount of breakage
Magnesium	Magnesium is essential for stabilizing ribosomes, for electrostatic shielding of DNA, and as a metal cofactor in enzymatic reactions[1].	Concentration
NADH	NADH is produced through the central metabolism in the majority of CFE systems and is required for ATP synthesis in lysates which utilize oxidative phosphorylation[55]. NADH can be measured via autofluorescence and may be a useful marker for metabolic activity.	Concentration
pH	pH affects the metabolism in lysate-based CFE systems and has been used to improve reaction yield or enzyme activity for pathway engineering[57].	Negative logarithm of the concentration of hydrogen ions
Phosphate	The accumulation of phosphate is known to inhibit protein expression and precipitates magnesium at neutral pH [58].	Concentration

4. Case Studies

The workshop included three case studies presented by invited speakers representing academia and industry, offering a broad perspective on advancing the use of CFE systems from the laboratory benchtop to a commercial product. As a professor at California Polytechnic State University and now as Director of R&D at Meso Scale Diagnostics, Dr. Javin Oza has worked to make cell-free platforms more accessible by improving each step of the CFE workflow. Dr. James Swartz's research group at Stanford University is responsible for many of the experimental breakthroughs in the field and has spun off companies seeking to bring cell-free products to market. One of these companies, Vaxcyte, was founded by Dr. Jeff Fairman, who spoke on the use of CFE systems in vaccine production.

Together, these case studies emphasized the ways in which the complexity of CFE systems impact their use in real-world settings. Approaches to reaction optimization and scale-up that incorporated DOE emerged as a recurring theme and an effective approach to tune reaction parameters and composition. Such approaches also help to address the common challenge of decoupling the effects of numerous experimental parameters and interpreting measurement results, especially as CFE is scaled up. Beyond experimental design, labware and equipment typically available in a biology wet laboratory for cell-based workflows must often be evaluated for its utility in CFE workflows. Especially for scaling reaction volumes towards liters and beyond, adapting existing labware and equipment or even developing entirely new tools for CFE systems remains an unmet challenge. Both the invited speakers and workshop participants agreed that CFE systems stand poised to enable development of biotechnologies that are prohibitive or impossible to engineer using traditional cell-based workflows. As the community strives to identify which applications present CFE systems with an "unfair advantage" over other systems, improved characterization and reproducibility will advance applications of CFE systems broadly and are key to bringing more CFE-based technologies to market.

5. Conclusions

Although stakeholder engagement informed the title and initial focus of the workshop on scale-up and automation for CFE systems, discussion during the workshop quickly indicated that measurements and standards needs require attention prior to, and as part of, working towards successful and reproducible CFE systems at all scales, with and without automation. In fact, the priority placed on measurements and standards clearly echoes the findings and recommendations presented in the report summarizing a related 2019 workshop[59]. This report highlights that scientific and technical challenges–rather than regulatory obstacles–present the most significant bottleneck to advancing CFE systems. For example, addressing the recommendations of this report will enable a deeper understanding of the impact of variability in CFE applications, which can inform regulators.

Beyond addressing regulatory concerns, overcoming the challenges discussed in this report will help to build understanding, capability, robustness, and trust in CFE systems, improve access and successful technology transition, and move the bioeconomy forward. Joint effort from academia, industry, and government to address the concerns and recommendations of this report can position CFE systems as a key tool and technology for biomanufacturing, complementary to cell-based systems.

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Appendix A. Abridged Agenda

Day 1: Thursday, February 8, 2024

9:00 AM **Registration**

9:30 AM **Welcome, scope, and deliverables**
Elizabeth Strychalski (NIST) and G. Sitta Sittampalam (NCATS)

10:00 AM **Plenary**
Raghavan Venkat (AstraZeneca)

10:30 AM **Break**

10:45 AM **Plenary**
Zachary Sun (Tierra Biosciences)

11:15 AM **Plenary**
Govind Rao (University of Maryland, Baltimore County)

11:45 AM **Panel**
Moderator: G. Sitta Sittampalam (NCATS)
Panelists: Raghavan Venkat (AstraZeneca), Zachary Sun (Tierra Biosciences), Govind Rao (University of Maryland, Baltimore County), Joel Welch (FDA)

12:30 PM **Working lunch in Working Groups**

1. Quality control for cell extracts *Lead: Fernanda Piorino (NIST)*
2. Reagent characterization *Lead: Jane Romantseva (NIST)*
3. Role of automation for optimization and scale-up *Lead: Thierry Masquelin and Alex Godfrey (NCATS)*
4. Analytical measurements and standards needs *Lead: Geoff Taghon (NIST)*

2:45 PM **Break**

Reports from Working Groups

3:00 PM *Moderator: Sitta Sittampalam (NCATS)*

3:10 PM **1.** Quality control for cell extracts

3:20 PM **2.** Reagent characterization

3:30 PM **3.** Role of automation for optimization and scale-up

4. Analytical measurements and standards needs

3:40 PM **Group discussion**
Moderator: Elizabeth Strychalski (NIST)

4:45 PM **Wrap-up, homework, and concluding remarks**
Jane Romantseva (NIST)

5:00 PM **Adjourn**

Day 2: Thursday, February 8, 2024

9:00 AM	**Registration**
9:15 AM	**Welcome and agenda** *Elizabeth Strychalski (NIST) and G. Sitta Sittampalam (NCATS)*
9:30 AM	**Case Study 1** *Javin Oza (Meso Scale Diagnostics)*
10:20 AM	**Case Study 2** *Jim Swartz (Stanford University)*
11:10 AM	**Case Study 3** *Jeff Fairman (Vaxcyte)*
12:00 PM	**Networking lunch**
1:00 PM	**Wrap-up** *Jane Romantseva (NIST)*
1:15 PM	**Adjourn**
1:30 PM	**(Optional) NCATS Automation tour** *G. Sitta Sittampalam (NCATS)*
3:30 PM	**(Optional) NCATS Automation tour wrap-up** *G. Sitta Sittampalam (NCATS)*

Appendix B. Registered Participant List

Ahern, Brooke
DEVCOM Chemical Biological Center

Alam, Khalid
Stemloop

Baer, R
Stemloop

Baker, Jeff
NIIMBL

Borhani, Shayan
Meso Scale Diagnostics

Cleveland, Megan
National Institute of Standards and Technology

Coburn, James
FDA

Croucher, Leah
NCATS

Fairman, Jeff
Vaxcyte

Feasel, Michael
DARPA BTO

Garcia, David
DEVCOM Chemical Biological Center

Gardner, Sean
NCATS

Godfrey, Alex
NCATS

Goh, Ee-Been
Sutro Biopharma

Greene, Shannon
DARPA

Jackson-Smith, Anton
b.next

Kelman, Zvi
IBBR

Klumpp-Thomas, Carleen
NCATS

Knauf, Kathrin
LenioBio GmbH

Lahiri, Sujoy
ATCC

Landgraf, Bradley
New England Biolabs

Lux, Matthew
DEVCOM Chemical Biological Center

Magnelli, Paula
New England Biolabs

Maheshwari, Akshay
b.next

Mangalampalli, Venkata
NCATS

Masquelin, Thierry
NCATS

Noireaux, Vincent
University of Minnesota

Norquist, Penny
BioMADE

Ottinger, Elizabeth
NCATS

Oza, Javin
Meso Scale Diagnostics

Piorino, Fernanda
National Institute of Standards and Technology

Rao, Govind
UMBC Center for Advanced Sensor Technology

Rezaei, Pegah
UMBC

Romantseva, Jane
National Institute of Standards and Technology

Rudnicki, Dobrila
NCATS

Rybnicky, Grant
NRL

Savino, Keith
Zymtronix

Schaffter, Sam
National Institute of Standards and Technology

Schiel, John
IBBR

Siegal, Dan
Ginkgo Bioworks

Sittampalan, G. Sitta
NCATS

Stoddard, Colby
Fermatix

Strychalski, Elizabeth A.
National Institute of Standards and Technology

Styczynski, Mark
Georgia Institute of Technology

Sun, Zachary
Tierra Biosciences

Sundberg, Chad
UMBC

Swartz, James
Stanford University

Taghon, Geoff
National Institute of Standards and Technology

Venkat, Raghavan
AstraZeneca

Venkat, Raghavan
AstraZeneca

Welch, Joel
FDA

Wintenberg, Molly
National Institute of Standards and Technology

Wolfe, Joshua
Georgetown University

Appendix C. Demographics and Working Groups

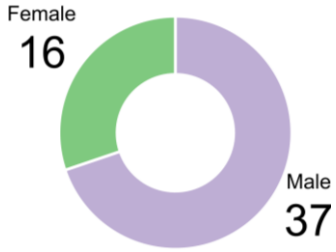

Fig. 3. Registered workshop participants by gender identity.

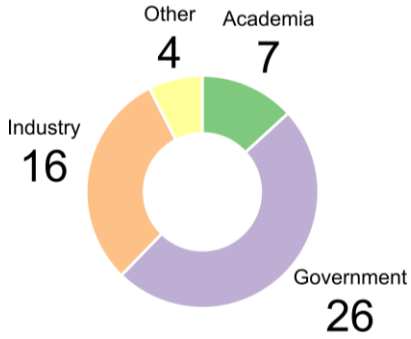

Fig. 4. Registered workshop participants by sector.

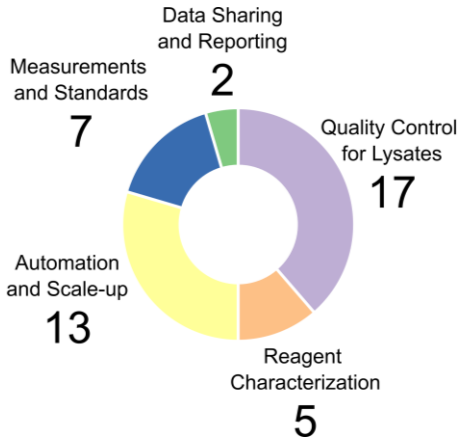

Fig. 5. Registered participants in each Working Group, collected during registration.