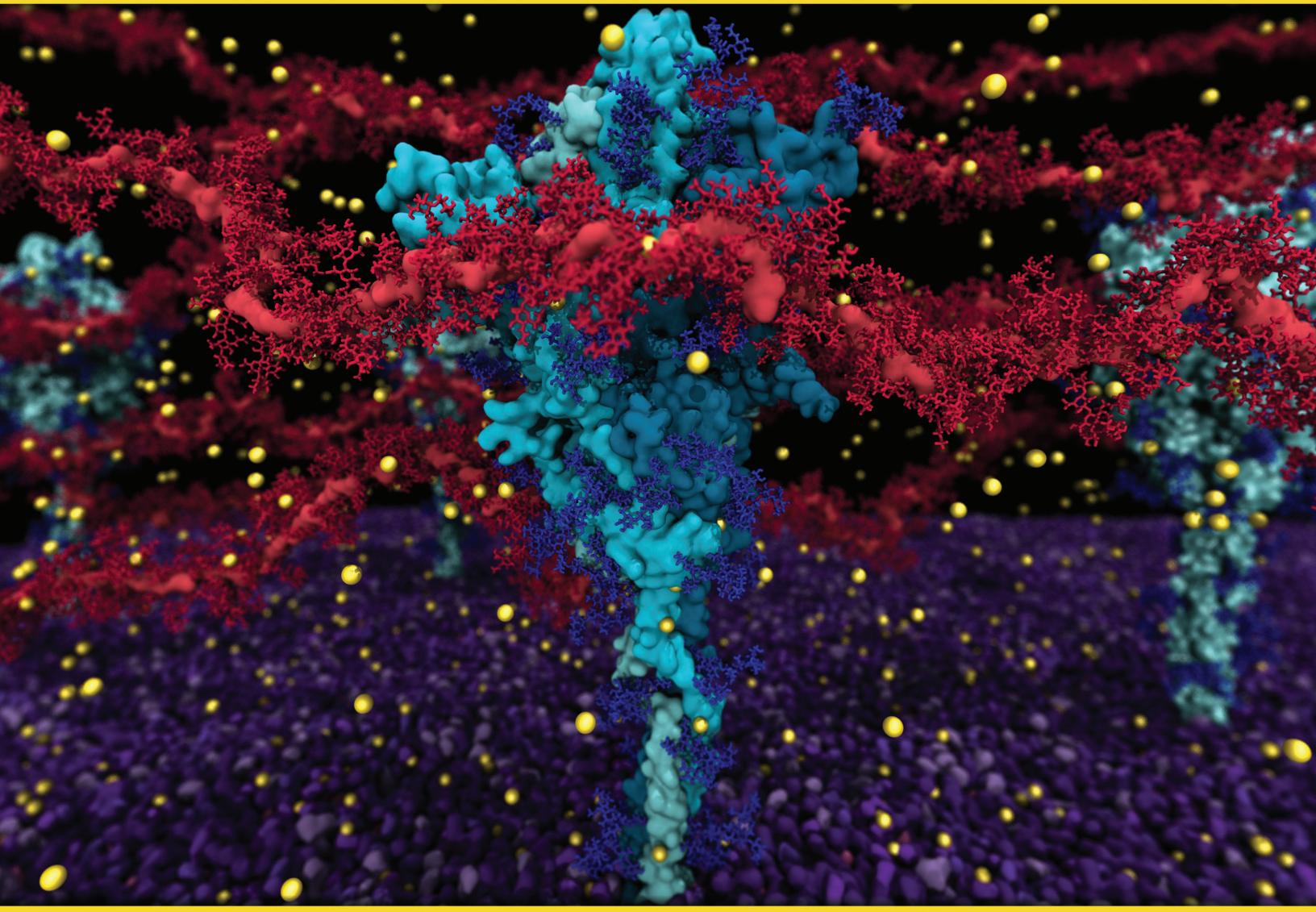
U.S. Department of Energy

Foundational Science for Biopreparedness and Response

Report from the March 2022 Roundtable



U.S. DEPARTMENT OF ENERGY | Office of Science

U.S. Department of Energy

Foundational Science for Pandemic Preparedness Roundtable

March 2022

About the Roundtable

In March 2022, the U.S. Department of Energy (DOE) Office of Science (SC) convened a virtual roundtable on "Foundational Science for Pandemic Preparedness" to identify the most important biopreparedness research areas and the unique roles SC could play in addressing future biological crises. The roundtable brought together participants from across the national laboratories, along with representatives from other governmental agencies and industry. Participants assessed U.S. biopreparedness challenges and identified five priority research opportunities and the specialized crosscutting capabilities needed to support biopreparedness studies at DOE national user facilities.

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Cover image: Molecular representation of delta SARS-CoV-2 spike proteins (cyan) surrounded by respiratory mucins (red) and calcium ions (yellow) within an aerosol. Viral membrane is depicted in purple. [Courtesy University of California, San Diego]

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Foundational Science for Biopreparedness and Response

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Executive Summary

In a rapid and impactful response to the COVID-19 pandemic—an event that has forever changed our perspective on biopreparedness—the U.S. Department of Energy (DOE) Office of Science established the National Virtual Biotechnology Laboratory (NVBL) in March 2020. Harnessing capabilities across all 17 DOE national laboratories, NVBL made critical advances by leveraging decades of DOE investments in basic science and experimental user facilities—including X-ray and neutron sources, leadership computing facilities, nanoscale science research centers, and biological characterization laboratories. This foundational research delivered the expertise and capabilities necessary to meet some of the greatest scientific challenges facing the research community during the pandemic (U.S. DOE 2021).

Given the inevitability of future biological crises, the nation must be better prepared to respond. This preparedness requires forward-leaning investments in relevant science and technology. Addressing future impacts on human, animal, and plant systems necessitates (1) building technologies that support surveillance and new diagnostics, (2) understanding the molecular mechanisms that lead to pathogenesis, (3) developing models that define disease transmission through our population and environment, and (4) exploring new materials that will make personal protective equipment and other countermeasures readily available and resistant to viral and bacterial contamination. Basic research focused on these topics, coupled with DOE's expertise and capabilities, will significantly improve our ability to quickly respond to future biological threats (see Fig. ES.1, p. v).

To identify the most important biopreparedness research areas, DOE's Office of Science convened a roundtable of participants from across the national laboratories, along with representatives from other governmental agencies and industry. Held in March 2022, the roundtable focused on understanding DOE's unique role in responding to future biological crises. Participants identified five priority research opportunities and the specialized crosscutting capabilities needed to support biopreparedness studies at DOE national user facilities. These research opportunities will drive a transformative research agenda to achieve the underlying science and technology advances needed for ensuring the nation's biopreparedness.

Priority Research Opportunities

1. Decode Pathogen Emergence, Evolution, and Host-Pathogen Dynamics in Real Time

Key Question: How do complex and dynamic biological systems interact with a host?

DNA and RNA sequencing capabilities for humans, plants, animals, and microbes have advanced significantly over the past three decades, but the ability to interpret these sequences has not kept pace. We also lack a complete understanding of the complex physical, chemical, and biological dynamics that occur when a pathogenic microbe interacts with a susceptible host. We therefore must transform biological science by discovering new principles and phenomena that will underpin the development of high-throughput analytical approaches capable of measuring and determining the dynamic networks that define pathogen-environment interactions, pathogen evolution, and host-pathogen interactions. Innovations are needed to enable real-time measurements that will enhance understanding of complex biological system interactions *in situ*. Such an understanding will accelerate the ability to continuously monitor biological systems and identify anomalies that can signal a developing crisis and help guide the response.

2. Build a Multiscale Understanding of Biomolecular Interactions to Catalyze Design of Targeted Interventions

Key Question: How do molecular interactions and vast biological networks give rise to cellular functions on physiological scales and co-evolution on ecological scales?

Preparing for the next biological crisis requires moving beyond understanding individual biomolecules, organelles, and microbes to understanding interactions of complex biological networks that drive cellular

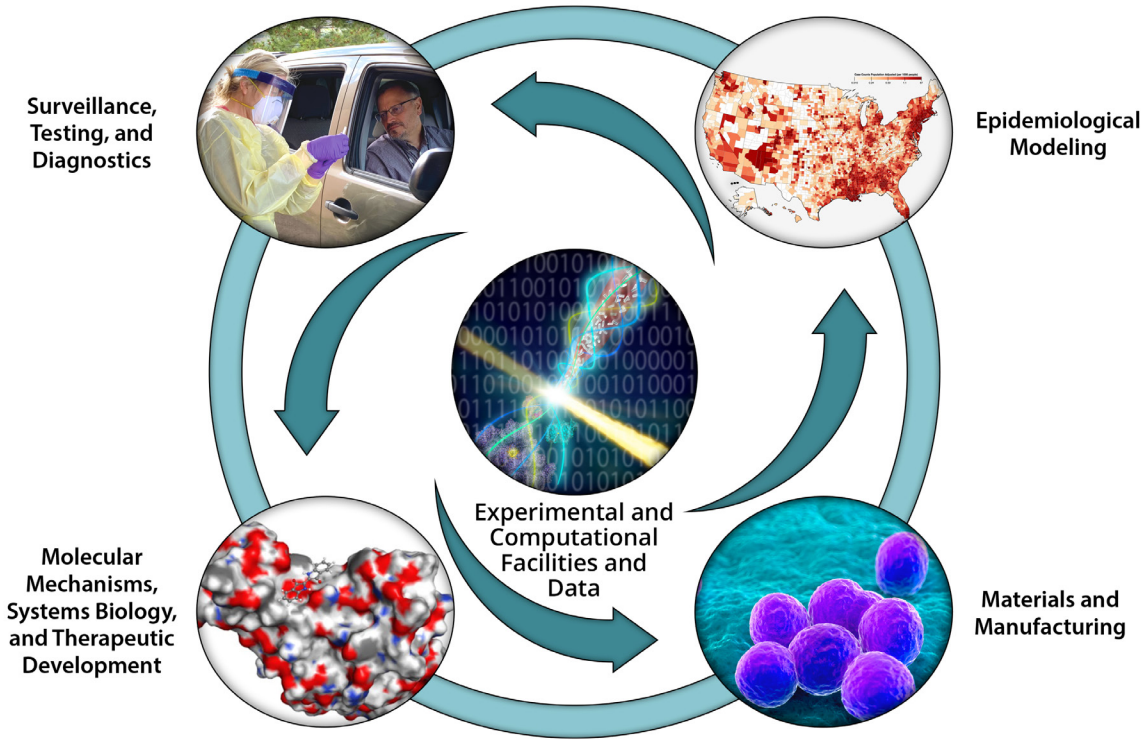

Fig. ES.1. Strengthening the nation's ability to prepare for and respond to future biological threats requires innovative, integrative, and collaborative research that intersects multiple disciplines and draws on diverse capabilities. DOE Office of Science experimental and computational user facilities are uniquely positioned to advance the foundational research for biopreparedness in four key areas outlined above.

[Image credits: Surveillance, testing, and diagnostics, Los Alamos National Laboratory; epidemiological modeling and molecular mechanisms, Oak Ridge National Laboratory; experimental facilities and data, SLAC National Accelerator Laboratory; materials and manufacturing, Getty Images.]

functions on physiological scales and co-evolution of microbes on ecological scales. Attaining this advanced knowledge will require unraveling interactions across vast numbers of molecules, with outcomes that manifest across many orders of spatiotemporal magnitude. In turn, wholly new approaches are needed to characterize molecules within the cell and understand their interactions with cell states, host physiology, and environmental factors. Success in this area will yield a massive reward: the technology to design and deliver new drugs, vaccines, and diagnostic prototypes in weeks rather than years or decades. In short, these developments would transform the nation's ability to prepare for, prevent, detect, respond to, and recover from biological incidents. Importantly, this undertaking would also provide broader insights into microbial evolution and function, which, in turn, would advance efforts to address climate challenges and support biomanufacturing and the bioeconomy.

3. Elucidate Multiscale Ecosystem Complexities for Robust Epidemiological Modeling

Key Question: How can complex and dynamic ecosystem interactions be captured in a framework of multiscale models?

Accurate representations of human-environment interconnections, particularly among the four key ecosystem components—human, animal, microbial, and Earth systems—are necessary to successfully model and quantify disease impact. Traditionally, epidemiological models have focused only on modeling disease dynamics within individual ecosystem components. Addressing this gap requires integrating

validated models across space, time, and disciplines, as well as assimilating real-time heterogeneous data streams to capture behavioral responses to environmental changes and interventions. Flexible, scalable, and disease-agnostic modeling frameworks will dramatically improve the ability to prepare for and quickly respond to emerging biological threats. Creating multiscale ecosystem approaches that leverage DOE computational facilities will help anticipate and reduce impacts to health, society, the environment, the economy, and infrastructure.

4. Exploit Biotic–Abiotic Interfaces to Accelerate Design, Discovery, and Manufacturing of Materials for Biopreparedness

Key Question: How do we understand, predict, and control biotic-abiotic interfaces in ambient conditions and across time scales?

The molecular details of pathogen-material interfaces are critical for understanding the environmental transmission of biological threats and, consequently, for protecting human health. Pathogen-surface interactions are extremely complex, and understanding them requires new characterization and modeling capabilities under ambient conditions and across time scales. Further, a fundamental understanding of biotic-abiotic interfaces is the foundation for developing the transformative technologies that will strengthen the nation's biopreparedness. Gaining such an understanding would enable, for example, the design of materials that control pathogen transport and leverage new antiviral and antimicrobial properties. This knowledge would also underpin creation of next-generation smart and wearable sensors to provide real-time pathogen detection. Finally, modular and distributed manufacturing will be critical for addressing supply chain issues during a biological event.

5. Accelerate Biopreparedness by Integrating Experimentation, Computing, and Globally Distributed Data

Key Question: How do we support innovative scientific research with integrated experimental, computational, and data capabilities?

The innovative research needed to accelerate scientific discoveries for biopreparedness requires a new paradigm that integrates experimental, computational, and data techniques. A systems approach is needed that combines complex heterogeneous data with autonomous experiments and real-time simulations. This approach would support efficient experiment-compute iterative processes and provide tools for data-to-knowledge transformations. Enabling scientific advances will also require new computational frameworks for model development along with secure and privacy-preserving data and metadata access, curation, and quality management. These foundational capabilities intersect with each of the priority research opportunities and, if realized, will accelerate breakthroughs in bioscience and biopreparedness.

Chapter 1

Introduction

In response to the COVID-19 pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the U.S. Department of Energy (DOE) Office of Science established the National Virtual Biotechnology Laboratory (NVBL) in March 2020. NVBL harnessed resources from all 17 DOE national laboratories and provided impactful advances in the fight against COVID-19 in five areas: (1) materials and manufacturing of critical supplies, (2) molecular design for therapeutics, (3) testing, (4) epidemiological modeling, and (5) viral fate and transport (U.S. DOE 2021). These advances were enabled by decades of DOE investments in basic sciences and experimental user facilities—including X-ray and neutron sources, leadership computing facilities, nanoscale science research centers, and biological characterization laboratories. This foundational research delivered the expertise and capabilities necessary to meet some of the pandemic's greatest scientific challenges.

NVBL's five project teams contributed significantly to the nation's COVID response. The COVID-19 Testing team developed new sampling and analysis technologies and supported U.S. Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) efforts to validate the effectiveness of commercial COVID tests. The Molecular Design team used both computational modeling and structural biology tools available at DOE user facilities to identify promising candidates for medical interventions. DOE light sources supported the development of FDA-approved therapeutics, including all three U.S. vaccines as well as antiviral drugs. The Epidemiological Modeling team provided local, state, and national decision-makers with information on disease spread and the impacts of restaurant and school closings and reopenings, along with other scenario-based analysis and mitigation planning. Working with industry, the Manufacturing team rapidly developed new materials and manufacturing processes that addressed shortages in face masks, test kit components, and ventilators. Finally, the Viral Fate and Transport team evaluated indoor and outdoor virus spread. Each NVBL project team also worked closely with federal, state, and local agencies, industries, and universities to address some of the most critical aspects of the COVID pandemic (U.S. DOE 2021).

Although DOE's Office of Science responded quickly to SARS-CoV-2 challenges, the nation's ability to preempt worldwide impacts from a future biological crisis requires significant enhancements. These advances can be achieved by building on NVBL's accomplishments and leveraging unique Office of Science strengths in the physical, computational, and biological sciences, as well as its suite of scientific user facilities. Preparing for potential impacts of a future biothreat on human, animal, or plant systems requires (1) building a knowledgebase that enables timely surveillance and new diagnostics, (2) understanding the molecular mechanisms that lead to pathogenesis, (3) developing models that define disease transmission through populations and the environment, and (4) exploring new materials that will make personal protective equipment and other countermeasures readily available and resistant to viral and bacterial contamination. Basic research focused on these topics, coupled with DOE's expertise and capabilities, will significantly improve our ability to quickly respond to future biological threats.

To identify the most important biopreparedness research areas, DOE's Office of Science convened a roundtable of participants from across the national laboratories, along with representatives from other governmental agencies and industry. Held in March 2022, the virtual roundtable on "Foundational Science for Pandemic Preparedness" focused on understanding DOE's unique role in addressing future biological crises. Participants identified five priority research opportunities and the specialized crosscutting capabilities needed to support biopreparedness studies at DOE national user facilities.

To prepare for the roundtable, DOE biodefense experts developed a technology status document (see Appendix D: Technology Status Document—Foundational Science for Pandemic Preparedness, p. 46) that summarizes current capabilities in biopreparedness and response and identifies technical bottlenecks. The virtual workshop commenced with plenary speakers from several governmental agencies, including the Defense Threat Reduction Agency, CDC, and National Institutes of Health. Speakers provided their perspectives on U.S. biopreparedness challenges and the most significant technology gaps that could be closed with fundamental research. Plenary sessions were followed by a two-week period of asynchronous virtual panel discussions identifying the most promising research opportunities in five key areas:

- **Panel 1:** Surveillance, Testing, and Diagnostics
- **Panel 2:** Molecular Mechanisms, Systems Biology, and Therapeutic Development
- **Panel 3:** Epidemiological and Event Modeling for Response and Recovery
- **Panel 4:** Materials and Manufacturing
- **Panel 5:** Crosscutting Team: Facilities and Data

The following chapters provide in-depth descriptions of the five priority research opportunities and additional roundtable outcomes.

Chapter 2

Surveillance, Testing, and Diagnostics

2.1 Introduction

Early recognition of a biological event is critical to mitigating its effects. Recognition includes two elements: (1) an initial indication that an unusual event is occurring, typically based on the observation of an anomalous pattern during surveillance, and (2) characterization of the event, often including identification of the causative pathogen (e.g., virus, bacterium, or fungus). Validated analytical methods, tools, and technologies for surveillance, testing, and diagnostics are foundational for both elements and therefore essential for changing the event's trajectory with earlier interventions (see sidebar, p. 4).

Current surveillance methods often rely on astute observations of host symptoms or behaviors. For example, a clinician might observe an atypical cluster of symptoms or see an uptick of patients. Based on these observations, the clinician might order a set of laboratory tests to help determine the cause. If a common illness, like influenza, is suspected, a point-of-care rapid diagnostic might be available to confirm or rule out a specific disease.

Transformational advances in genome sequencing, enabled by the Human Genome Project and subsequent genomic studies on a wide range of organisms, have provided the basis for many tests and diagnostics used today. Most are laboratory-based or otherwise poorly suited for widespread use, so they are not commonly used for surveillance. As such, coverage and timely identification of an unusual biological event's onset are limited. The response to the COVID-19 pandemic demonstrated significant advancements in our ability to sequence host, pathogen, and environmental genomes and design genome-based tests; however, simply knowing sequences is not sufficient. We need more detailed knowledge of the myriad physical, chemical, and biological processes that occur when a pathogenic microbe interacts with a susceptible host, such as how sequence data can predict emergent properties of host-pathogen interactions that can affect a new variant's medical impact or its long-term effects.

Addressing these knowledge gaps requires (1) understanding the underlying molecular-level processes that define the complex networks involved in host-pathogen interactions, pathogen-environment interactions, and pathogen evolution and (2) developing new analytical approaches to characterize these dynamic networks. Expanding our fundamental knowledge of the principles and phenomena that define pathogens, host response, and host-pathogen dynamics will allow us to not only anticipate pathogen emergence and evolution, but also predict virulence and host immune response. This information will facilitate new detection approaches that are pathogen-agnostic and applicable to multiple host species. Together, these advances will transform our ability to identify and therefore mitigate a future event before it escalates into a pandemic.

2.2 Priority Research Opportunity

Goal: Decode pathogen emergence, evolution, and host-pathogen dynamics in real time

DNA and RNA sequencing capabilities in humans, plants, animals, and microbes have advanced significantly over the past three decades, but the ability to interpret these sequences has not kept pace. We cannot predict the change in infectivity or seriousness of a virus from sequence data. Further, we lack a fundamental understanding of the complex physical, chemical, and biological dynamics that occur when a pathogenic microbe interacts with a susceptible host. We must discover novel principles and phenomena that will underpin the development of new analytical approaches capable of measuring and determining the dynamic networks that define pathogen-environment interactions, pathogen evolution, and host-pathogen interactions. Such an understanding will accelerate the ability to continuously monitor biological systems and

Importance of Early Intervention and Ecosystem Interactions

Enabling earlier interventions improves response efficacy and lessens a biological event's impact. Most interventions are more effective when delivered earlier (see Fig. 2.1), whether they are specific (e.g., vaccination) or broad (e.g., masking or administering prophylactics, such as antifungals and antibiotics for humans, animals, and plants). Additionally, timely sample collection and analyses provide opportunities for earlier interventions. As we learned from the COVID-19 pandemic response, effective public health support and related decision-making rely on accurate and highly sensitive characterization of the outbreak—including pathogen variant, host response, and countermeasure effectiveness.

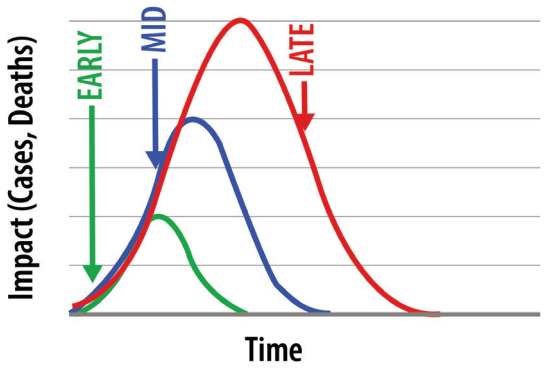

Fig. 2.1. Impacts of a biological event become more severe the later interventions occur.
[Image credit: Los Alamos National Laboratory]

Hosts range from simple organisms (e.g., bacteria, virus, and fungi) to higher organisms (e.g., plants, animals, and humans). Scientific characterization of a host as normal and healthy versus abnormal might require assessing a network of peer hosts, pathogens, and symbiotic and commensal organisms. Essential information about pathogens and hosts must be extracted from a representative

Continued on next page

identify anomalies that can alert us to a developing crisis and help guide our response.

2.3 Scientific Impact

Understanding the fundamental physical, chemical, and biological processes and dynamic interactions between microbes and multicellular organisms—including host-pathogen interactions, pathogen-environment interactions, and pathogen evolution—is critical for biopreparedness. We require insights into the molecular-level interactions of pathogenic microbes with hosts and the environment, including a background of diverse microbes. Gaining this knowledge will require new analytical approaches and provide the foundations for developing new analytical tools able to measure *in situ* and real-time dynamic interactions of microbes and host organisms, with each other and with the environment. The new knowledge and tools gained from this research will lead to major advances in understanding of biological systems under normal and perturbed conditions with relevance to many applications, including biopreparedness.

Molecular-level understanding may also enable new capabilities in sensing and analytical methods that are pathogen- and host-species agnostic. Such advances would significantly enhance our ability to detect and respond to unanticipated pathogens. Discovery of novel scientific principles and phenomena that define pathogens, host response, and host-pathogen interactions will enable proactive identification and forecasting of emerging threats in time to inform pandemic response and impact pandemic trajectory.

Continued from previous page

environment that includes a complex network of dynamic interactions among viruses, bacteria, fungi, and multicellular organisms. Interactions are modulated by environmental factors, like temperature and humidity. Host susceptibility is an evolving state in the network and might change due to environment, fatigue, stress, previous exposure, co-infection, and societal factors, among others (see Fig. 2.2).

Decoding pathogen emergence, evolution, and host-pathogen dynamics requires new analytical tools that integrate multiple data types—such as omics, imaging, and measurements of other chemical, physical, and biological phenomena that reveal the cascade of events occurring when microbes and host organisms interact across various time and length scales. For example, populations and the environment must be monitored to characterize normal conditions, identify anomalies that might indicate an impending crisis, and detect the molecular markers for an anomaly's presence in physiological samples. Data from these measurements, in turn, must be analyzed to provide insights into pathogen interactions with hosts and the environment, so we can predict the impact of a pathogen on a host, forecast pandemic trajectories, and enable effective and timely interventions.

Fig. 2.2. Host susceptibility is an evolving state. Scientific characterization of a host might require assessing a network of peer hosts, pathogens, and symbiotic and commensal organisms. Susceptibility might evolve due to environment, fatigue, stress, previous exposure, co-infection, and societal factors.

Improved understanding of basic molecular mechanisms may also lead to discovery of environmental or population-scale correlations that enable global surveillance. A major innovation towards optimal surveillance will build on measuring and understanding background and identifying anomalies at their leading edge and at detection levels relevant to infectious or health effects. Robust predictive signatures will allow us to make unprecedented background measurements and to define what is normal or expected in complex, coupled biological systems. Furthermore, discovery of molecular-level mechanisms will provide critical information on host health status and pathogen evolution. Such information can be used to guide a multiscaled pandemic response—from individual to global—and to inform countermeasure development (e.g., vaccines, drugs, and antibodies, described further in Ch. 3: Molecular Mechanisms, Systems Biology, and Therapeutics, p. 8).

Ultimately, when we understand the correlative signals that define functions of and interactions between microbes, organisms, and the environment, we can begin to predict and harness these phenomena to benefit the Earth. As such, knowledge gained from this priority research opportunity extends beyond strengthening the nation's pandemic preparedness. It will also provide a more complete and accurate understanding of Earth's ecosystems and benefit broad expanses of the bioeconomy that can lead to profound impacts in agriculture; biofuels production; bioremediation; carbon, nutrient, and elemental cycling; climate remediation; medicine; and metabolic engineering.

2.4 Scientific Challenges

Current approaches for surveillance, testing, and diagnostics rely on prior knowledge of disease symptoms in a host (e.g., medical surveillance) or measurement of a known pathogen's static property (e.g., polymerase chain reaction tests and immunoassay-based diagnostics). Improvements to these approaches are limited by the following fundamental challenges: (1) understanding how a pathogenic microbe interacts with a host; (2) characterizing what differentiates a pathogenic microbe from a nonpathogenic microbe; and (3) identifying the physical, chemical, and biological processes that occur at early stages of interaction. These interactions are complex and difficult to measure. However, if we can overcome this barrier and elucidate pathogen emergence, evolution, and host-pathogen dynamics, we can transform surveillance and response approaches to a future biological event and, ultimately, prevent it from escalating into a pandemic.

Further, enabling the predictive understanding of microbial interaction with multicellular organisms will enable investigations into key questions, such as: What are the measurable differences between pathogen categories (e.g., virus, bacterium, and fungus) in a host and in the environment? How can we track disease progression in a host? How can we track and predict the evolution of a pathogenic microbe in real time?

Armed with this knowledge, we may be able to understand and predict zoonotic leaps, which occur when pathogenic microbes jump from one species to another. Moreover, molecular-level understanding of infections could facilitate the identification of universal or near-universal process features, such as those associated with known human cell receptors. This discovery, in turn, could lead to the holy grail of surveillance, testing, and diagnostics methods: disease-agnostic surveillance tests able to identify the presence of a pathogen of any kind.

Improved pathogen detection and characterization approaches will also require an increased scientific understanding of emergent properties resulting from interactions of multiple microbes and/or multicellular organisms under both normal and perturbed conditions. New approaches for elucidating the myriad processes involved in infection are needed at a range of time and length scales—from real-time, *in situ* measurements in single cells to high-sensitivity characterization of infection in multicellular organisms. Approaches that combine multiple modalities of analyses will provide the critical information-rich characterization needed to reveal detailed understanding of how physical, chemical, and biological dynamics combine to produce the emergent properties that comprise the phenome (i.e., the complete set of characteristics of the individual organism).

A common theme across the scientific challenges lies in combining data and mechanistic understanding with models that enable predictions of individual organism response from the molecular to the cellular or multicellular level. These analytical approaches will require new methods for data interpretation and assimilation of large datasets. Current capabilities in machine learning, deep learning, and artificial intelligence must be expanded to enable detailed understanding of the myriad interactions among pathogenic microbes, potential hosts, and the environment. New approaches also will be needed for data validation, curation, and distribution to the broader research community to improve the accuracy, reproducibility, quality, and validity of systems biology models.

The overall goal of this priority research opportunity is to develop a predictive understanding of the full dynamic microbe-host-environment network so that surveillance is an effective early diagnostic of potential biological events. Our current understanding falls far short of what is needed. Advancing our understanding of the physical, chemical, and biological dynamics of the interactions of multiple microbes and/or multicellular organisms will enable us to identify novel phenomena and signatures that will underpin new rapid, high-sensitivity, and robust approaches for pathogen detection and characterization.

Chapter 3

Molecular Mechanisms, Systems Biology, and Therapeutics

3.1 Introduction

The arrival of SARS-CoV-2 revealed both strengths and weaknesses of our societal defenses against pandemic disease. Vaccines and antivirals were developed 10 times faster than those for other diseases, yet the death toll in the United States alone has exceeded 1 million, and disabilities due to "long COVID" and intermediate disease states are expected to impact our society for decades to come. Technology has advanced our protections and protective strategies but not enough; the future requires that we accelerate our rate of response at least tenfold again to prevent mass death and disability from possible future biothreats. Achieving that pace will require fundamentally new technologies and advances in basic science.

The mRNA vaccines that attenuated COVID-19 impacts owe their origins to the Human Genome Project. Led by DOE and the National Institutes of Health, this biology "moonshot" at the end of the previous millennium incubated technologies required to code in the language of nature: DNA, RNA, and proteins. Our capacity to read and synthesize the structures that give rise to function allowed us to use our own cells to construct viral proteins against which to develop immunity and resistance. Also, knowledge of protein structures enabled the development of effective antivirals to inactivate essential proteins in the virus. Newfound technologies to scale bioreactors delivered these advancements nationally, and then globally, at speeds previously inconceivable.

In the coming century, however, we must move beyond understanding individual molecules and proteins to understanding interactions and the vast biological networks that give rise to cellular functions on physiological scales and to coevolution on ecological scales. This will require a sustained effort to elucidate molecular interactions across scales that will then enable us to characterize molecules in subcellular contexts and associated with cell states, host physiology, and environmental factors. Decoding the molecular foundations of cellular and organism function will require unraveling interactions among vast numbers of possible molecules with outcomes that manifest across six orders of magnitude of spatiotemporal scales. This undertaking is massive, but the rewards would be equally outsized—drugs and vaccines developed in weeks and a transformation in the nation's disease-fighting ability and overall biopreparedness.

Such a transformation requires a research architecture that will reveal the foundations of interspecies and viral interactions at ecosystem scales. This architecture will revolutionize our understanding of natural ecosystem interactions—from molecules to organisms to communities—and reveal the tools for synthetic biology that will become foundation for a circular bioeconomy. In times of an emerging biothreat, we will be ready to pivot and effectively respond with unprecedented speed.

3.2 Priority Research Opportunity

Goal: Build a multiscale understanding of biomolecular interactions to catalyze design of targeted interventions

The holy grail of molecular biology is a map from molecular interactions to dynamic models of cells and biological phenotypes. Multiomics technologies—along with structural biology tools, especially X-ray crystallography—have given us a "parts list," while cryo-electron microscopy (cryo-EM) and other imaging tools have made it possible to study molecules in their natural contexts, albeit typically at cryogenic temperatures. Protein interaction and reaction networks provide insights into gene and molecular functions, and metabolic

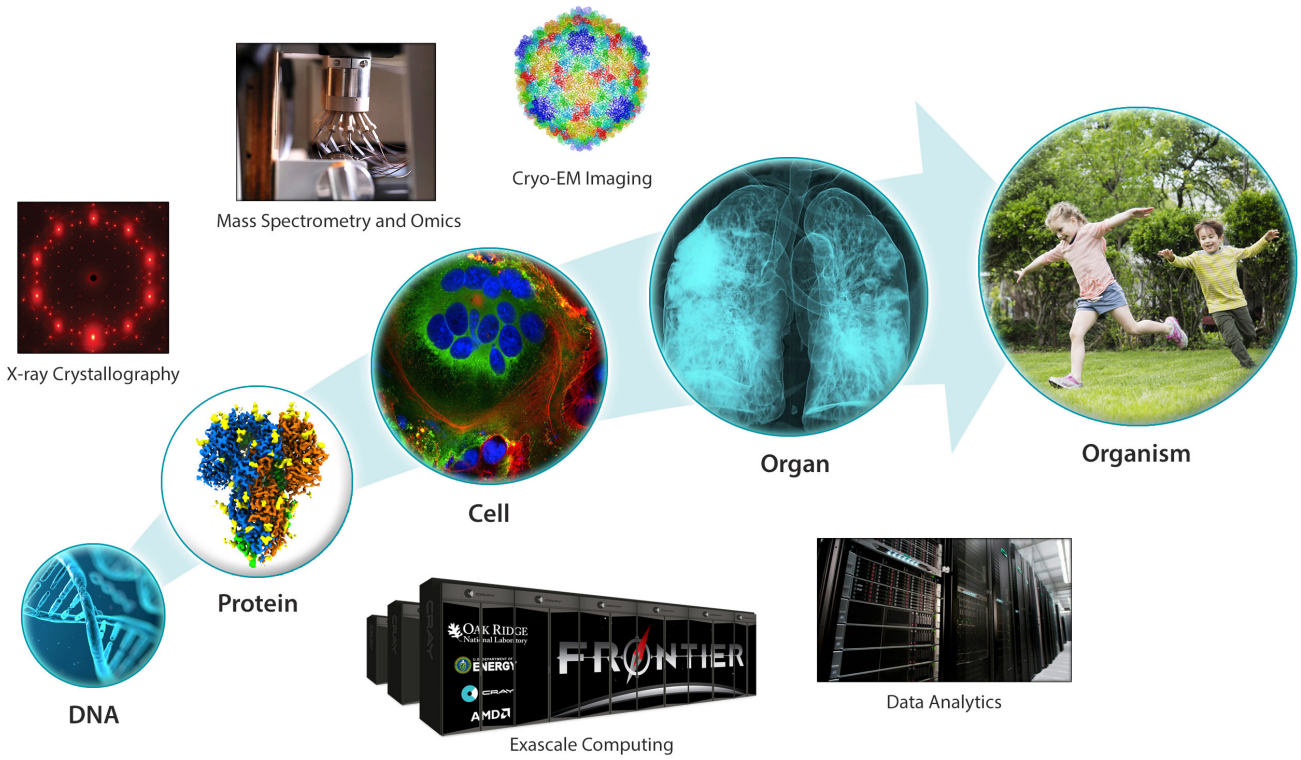

Fig. 3.1. Future biopreparedness will rely on advanced computing capabilities and integration of experimental data across spatiotemporal scales to create an understanding of biomolecular interactions.

[Image credits: X-ray crystallography, Ames Laboratory; mass spectrometry omics, Environmental Molecular Sciences Laboratory; DNA and organism, Getty Images; cryo-EM, SLAC National Accelerator Laboratory; see also Hryc, C. F., et al. 2016. *PNAS.* DOI:10.1073/pnas.1621152114. Protein reprinted under a Creative Commons License (CC BY 4.0) from Zhang, K., et al. 2020. "A 3.4-Å Cryo-Electron Microscopy Structure of the Human Coronavirus Spike Trimer Computationally Derived from Vitrified NL63 Virus Particles" *QRB Discovery*, **1**, E11. DOI: 10.1101/2020.08.11.245696. Cell, Mauro Giacca; see also Scudellari, M. 2021. *Nature.* DOI:10.1038/d41586-021-02039-y. Organ, Rajaaisya/Science Photo Library. Exascale computing, Oak Ridge National Laboratory. Data analytics, Argonne National Laboratory.]

models enable the study of dynamics. However, the relative quality of information across these resources varies widely. For example, protein structures, at least for crystallizable components, can achieve high-quality, atomic-resolution data, but reaction networks are of low quality or are unavailable outside of a handful of model organisms. Further, even when reaction networks are identified, they are often grossly incomplete. Similarly, metabolic models often make profound simplifying assumptions in the absence of data to serve as quantitative constraints. Even the comparatively simple task of ascertaining the native substrate of an enzyme of unknown function is currently a massive undertaking, as is the optimization of an enzyme for a target function. As a result, predicting the molecular evolution of proteins in environmental contexts is largely intractable. Coordinated, multimodal investigations with the express purpose of elucidating molecular interactions and their impacts on cellular and organismal functions are needed to radically accelerate the state of knowledge for the biological sciences. Addressing these challenges will require a research strategy that tightly integrates computation and experiment, engineers synthetic systems that recapitulate phenotypes and causal biological pathways, and designs molecular and biomolecular reagents that modulate targeted biological responses (see Fig. 3.1, this page).

3.3 Scientific and Biopreparedness Impact

The impact of building a multiscale understanding of molecular interactions and mechanisms will be a seismic shift in how we model, predict, and manipulate biological systems. Examples include (1) comprehensive cell models that propagate molecular interactions across spatiotemporal scales, thereby linking processes to the emergence of phenotypes and organismal states (molecular biology); (2) a predictive understanding of host-externality interactions at the molecular scale that reveals how organisms sense and interact with their biotic and abiotic environments (molecular ecosystems biology); and (3) new translational pipelines to predict, design, and implement molecular controls of biological systems at organismal and ecological scales (synthetic biology).

Molecular maps linking genome to phenome via interactions will provide a deep understanding of how biological structures operate in cellular and subcellular contexts to give rise to or disrupt homeostasis and ultimately determine physiological responses. These maps will enable the prediction of biological perturbations, as well as the optimization of microbes, plants, and communities, to produce target chemical and biological compounds—a new generation of biofoundry that realizes the potential of DOE's long-term investment in synthetic biology. Part of this revolution will depend on identifying the parameters and system features that control and modulate physiological and ecological states. Potential exists beyond bioreactors to develop control systems for ecosystem services that could drive natural and managed ecosystems toward enhanced utility.

We already have the computational capacity to model interorganismal protein evolution—the convergent evolution of proteins between, for instance, a symbiote and its host. However, such tasks are enormously expensive, and developing coevolutionary models (including enzyme targets and activities and protein-protein interactions) and translating them to quantitative, dynamic systems models will push the boundaries of what is possible with high-performance computing.

The research infrastructure required for this work will have profound impacts on foundational science as well as on our national capacity to not only rapidly respond to new and emerging threats, but also anticipate future threat landscapes. Such models will provide new capabilities to predict the evolution of potentially pathogenic organisms and viruses and identify the potential for zoonotic hops or co-option by nefarious actors for bioattacks. These models will also facilitate the rapid development of reagents to restore systems to normal function after external attack. For example, the ability to predict the evolution of a virus in its interactions with the host and the environment would be incredibly powerful, allowing researchers to intercept the process with targeted therapies before a new variant takes over.

3.4 Scientific Challenges

The goal of this priority research opportunity is to build a multiscale understanding of biomolecular interactions and mechanisms to elucidate the causal relationships between microscale biological pathways and macroscale biological phenotypes. This new knowledge will, in turn, catalyze discovery and design of biomolecular systems and targeted interventions. This research will also create high-resolution, high-throughput, and high-accuracy pipelines that integrate multiscale, multimodality observations; use synthetic biology, nanobiology, and chemical biology tools for precise perturbation of model systems; and incorporate rapid feedback between computational prediction and experimental exploration. High-fidelity synthetic systems should capture cell-organism and interorganism interactions and should safely enable high-throughput multiomics and forward genetics. Achieving this priority research objective will require surmounting scientific challenges across multiple categories.

Scientific Challenge 1: Designing and Engineering Synthetic Systems that Reproduce Key Aspects of Biological Systems

Synthetic systems encompass both biologically relevant experimental models and computational digital twins. The goal for both is to establish causal relationships between subcellular features and phenotypic hallmarks of individual organisms and the mutual responses between organism and the environment. Information gleaned from synthetic systems research

allows us to understand how interactions propagate across scales and give rise to physiology.

Current experimental synthetic systems cover multiple scales. Some of this research focuses on cell-based models, which contain only limited components of living cells (Salehi-Reyhani et al. 2017). Examples include liver microsomes (Asha and Vidyavathi 2010) and the pseudovirus models that have enabled important SARS-CoV-2 research without requiring biosafety level (BSL) 3 or 4 containment facilities (Li et al. 2018; Dieterle et al. 2020). Other experimental synthetic systems research explores systems as complex as organoids (Kim et al. 2020) or induced pluripotent stem cells (iPSC; Shi et al. 2017), which aim to model complex organ and tissue structures.

While much progress has been made in the ability to create such model systems, scientific challenges remain in translating insights from synthetic systems into an actionable understanding of biology in organisms, communities, and environments. One significant challenge is that multiple mechanisms and pathways can lead to a specific observed phenotype, but we lack the ability to predict which mechanisms are at play. Even in the case of well-characterized synthetic systems, such as liver microsomes to characterize metabolites, differences in how samples are prepared can lead to differences in how results should be interpreted (Wang et al. 2018). The challenges are multiplied in more complex synthetic systems, such as iPSCs. For example, stem cells have been successfully induced to differentiate into pancreatic islet cells that exhibit the physiologically relevant phenotype of glucose-responsive insulin secretion. However, large differences were observed in transcriptomics and metabolic pathways between the synthetic system and primary adult human islet cells (Balboa et al. 2022).

The world-leading computational capabilities of DOE's national laboratories provide a potential path for navigating the challenge of bridging complexities across scales from molecular mechanisms to biological phenotype through digital twins. Computational digital twins leverage machine learning, mechanistic, and systems models to adaptively integrate emerging data into a framework of extant knowledge and to guide experimentation with reliable assessment of the biological consequences of internal pathways and external factors (Filippo et al. 2020; Tellechea-Luzardo et al. 2020; Möller and Pörtner 2021). Such models are data hungry and require generating large amounts of data across scales in relevant synthetic and actual systems. These models must therefore be tightly coupled to the synthetic systems described above and to the structural, dynamic, and probe-molecule challenges described below. Further, these computational digital twins should be constructed in ways that allow us to elucidate causal relationships between metabolic pathways and biological phenotypes. However, multiple research and methodological advancements are needed to develop causal reasoning in computational models.

Scientific Challenge 2: Probing the Structure and Dynamics of Molecular Interactions in a Biologically Relevant Context

The synthetic systems described above would enable understanding of biological processes at cellular, organismal, and ecosystem scales. Developing causal relationships at atomic scales on the other hand will require building a subcellular understanding of proteins and other biomolecules in context, determining protein structures in native or native-like environments, and delineating how structures interrelate dynamically in metabolic contexts to give rise to system behaviors and biological phenotypes. The scientific challenge is to measure complex interactions with extreme structural and biochemical precision and to map those interactions of subcellular components within organisms and at the interface between organisms and their abiotic environments. A multiscale bioimaging capability is essential to address this scientific challenge.

The national laboratories have pre-eminent capabilities in imaging technologies through ongoing research and their user facilities, including X-ray light sources, neutron-scattering facilities, nanoscale science research centers, the Joint Genome Institute, and the Environmental Molecular Sciences Laboratory (see sidebar, p. 12). These resources provide multiple technologies for interrogating structure and dynamics, including (1) tomography, microscopy, and crystallography using fluorescence, X-rays, neutrons, and electrons; (2) single-cell and bulk genomic, transcriptomic, proteomic, and metabolomic measurements using mass

DOE User Facilities Are Key to Biopreparedness

DOE Office of Science national user facilities host large-scale, valuable scientific capabilities that universities and industrial research laboratories lack the resources to design, build, or operate. These capabilities are made available to researchers through a peer-reviewed proposal system. The facilities include advanced supercomputers, X-ray and neutron sources, high-resolution electron microscopy and imaging techniques, nanoscience laboratories, particle accelerators, high-power laser systems, biological characterization tools, and test beds for new carbon-free energy technologies.

In addition to enabling scientific discoveries, these world-leading user facilities are ideally positioned to help deliver rapid response to address threats and disasters. In the case of COVID-19, the user facilities quickly responded in areas such as modeling of disease spread and community response, development of new testing protocols, and perhaps most impactfully, development of vaccines and identification of potential drug candidates.

Understanding the structure of a virus is the first step in finding treatments or vaccines. Between January 2020 and September 2021, 30% of the 1,574 structures related to coronavirus research released globally by the Protein Data Bank utilized DOE light and neutron user facilities. One example (see Fig. 3.2) is the crystal structure of the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2 in complex with human angiotensin-converting enzyme 2 (ACE2). This structural understanding is critical to designing SARS-CoV-2 RBD vaccines.

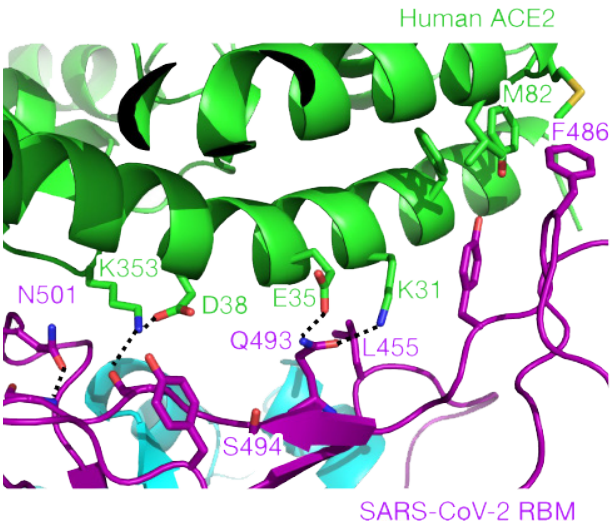

Fig. 3.2. Atomically precise models of protein structure are key to understanding the function of proteins and the design of therapeutics. Here X-ray crystallography reveals the structure of the SARS-CoV-2 spike protein domain 2 (purple) bound with the ACE2 human protein (green).

[Image credit: Reprinted by permission from Springer Nature from Shang, J., et al. 2020. "Structural Basis of Receptor Recognition by SARS-CoV-2," *Nature* **581**, 221–24. © 2020.]

spectrometry, nuclear magnetic resonance spectroscopy, and other analytical techniques; and (3) cryo-EM and cryo-electron tomography. Continued fundamental research will move beyond characterization of biological macromolecules in solution and will:

- Determine structures and interactions of complex macromolecules in their native environments under *in vivo* conditions, such as at room temperature and in the robust synthetic systems described above.
- Build dynamic models of structures and interactions between structures in metabolic contexts.
- Enable accurate prediction and uncertainty quantification for biomolecule structures in solution and in cellular or subcellular contexts.

- Characterize the structural and dynamic consequences of external perturbations of native environments and synthetic systems.

Cutting-edge computational capabilities at the national laboratories will enable data integration across experimental modalities for a comprehensive, multifactorial characterization of the structure, dynamics, and functional consequences of biomolecules at atomic scales.

Scientific Challenge 3: Generating Tools and Molecules that Can Probe, Modulate, or Interfere with Biological Pathways and Phenotypes

In tandem with the two scientific challenges described above, the priority research objective proposed in this chapter requires tools and molecules that can modulate synthetic systems, characterize the structural and dynamic consequences of that modulation, and translate resulting discoveries into an actionable understanding of biology.

These tools will enable manipulation of the internal genetics of synthetic systems and their interactions with external molecules, with the ultimate goal of being able to predict and control biological outcomes from genetic and molecular manipulations. A range of genome-editing techniques can assist in deconvolving the connection of single phenotypes to multiple biological pathways (Khalil 2020; Li et al. 2020). Additionally, a diverse range of molecular probes can automate synthesis of chemicals (Collins et al. 2020; Shen et al. 2021), oligosaccharides (Wen et al. 2018), and oligonucleotides (Song et al. 2021), while chemical biosynthesis (Prather et al. 2008; Singh et al. 2016) and flow chemistry (Ley 2012; Plutschack et al. 2017; Gudi et al. 2020) can provide more sustainable techniques for making these molecular probes. These automated biosynthesis and flow technologies are—or are becoming—standards in the toolkit for making molecules, but scientific challenges persist with each technology and with using the technologies to generate a diverse pool of molecules that can best interrogate synthetic and actual biological systems. Building this capability will not only enrich our understanding of biology, but will also enable our capacity to pivot to synthesis at scale (both in number and quantity) in response to an emerging biothreat.

Taken together, progress toward each of these scientific challenges will build an enduring capability that accelerates DOE aspirations for genes-to-ecosystem understanding, synthetic biology, and a circular bioeconomy. Such advancements will also ensure that national laboratories have the capabilities to pivot in response to the next biological crisis even more rapidly than for COVID-19, achieving a ten- or hundredfold acceleration beyond our current capacity.

Chapter 4

Epidemiological and Event Modeling for Response and Recovery

4.1 Introduction

Historically, epidemiological models have been used to understand causes of disease spread (Manheim et al. 2016) and to quantify impacts of different intervention strategies. More recently, these models have been used to forecast disease spread (Biggerstaff et al. 2016). The most basic approach to epidemiological modeling consists of breaking the population into susceptible, infectious, and recovered individuals, or SIR models, and assuming different rates at which individuals move through the system (Hethcote 2000).

Computational advancements enable large-scale agent-based simulations (Germann et al. 2006, 2019; Ozik et al. 2021) that capture millions of individuals interacting in various settings, from neighborhoods to cities and countries. These simulations are subsequently analyzed for impacts of heterogeneous assumptions on disease transmission and effects of individual decisions on aggregate behavior. Additionally, statistical models analyze trends and enable short- and long-term forecasts for many diseases (Dixon et al. 2022).

While most of these modeling approaches were built with the intention to understand disease spread, decision-makers are turning to epidemiological modelers for real-time decision support. As such, current limitations in epidemiological modeling approaches require scientific advancements to more accurately capture the complex interactions and processes contributing to disease spread and to enable on-demand operational models (Desai et al. 2019). Achieving these advancements necessitates large-scale coordinated efforts to provide infrastructure for global data stream collection, validated ensembles of multiscale ecosystem models, and robust results with quantified uncertainty.

The technical challenges in realizing this vision are:

- Exploiting and expanding high-performance computing (HPC) infrastructure to enable global collaboration.
- Integrating models of humans, animals, Earth systems, and microbial communities.
- Developing models that capture emergent behavior and adaptation.
- Exploiting heterogeneous data streams and standardization of data collection approaches.
- Combining data- and model-driven approaches to capture the mechanistic dynamics of these systems.
- Incorporating uncertainty quantification (UQ) techniques into integrated computational models.

Advancements in scientific foundations of epidemiological forecasting will enable lasting impacts on our nation's preparedness and response capabilities. Improving data-driven modeling approaches—for disease surveillance, human population density, mobility, host and environmental susceptibility, pathogen transmissibility, and healthcare capacity—will ensure timely anticipation of disease dynamics and adaptation of mitigative measures that will minimize consequential impacts to our social, economic, and critical infrastructures.

Using scalable, HPC infrastructures and interdisciplinary expertise to develop such modeling capabilities across a broad and reliable data source range would create a flexible data-to-model-to-knowledge system that translates scientific knowledge for real-time operational environments on demand.

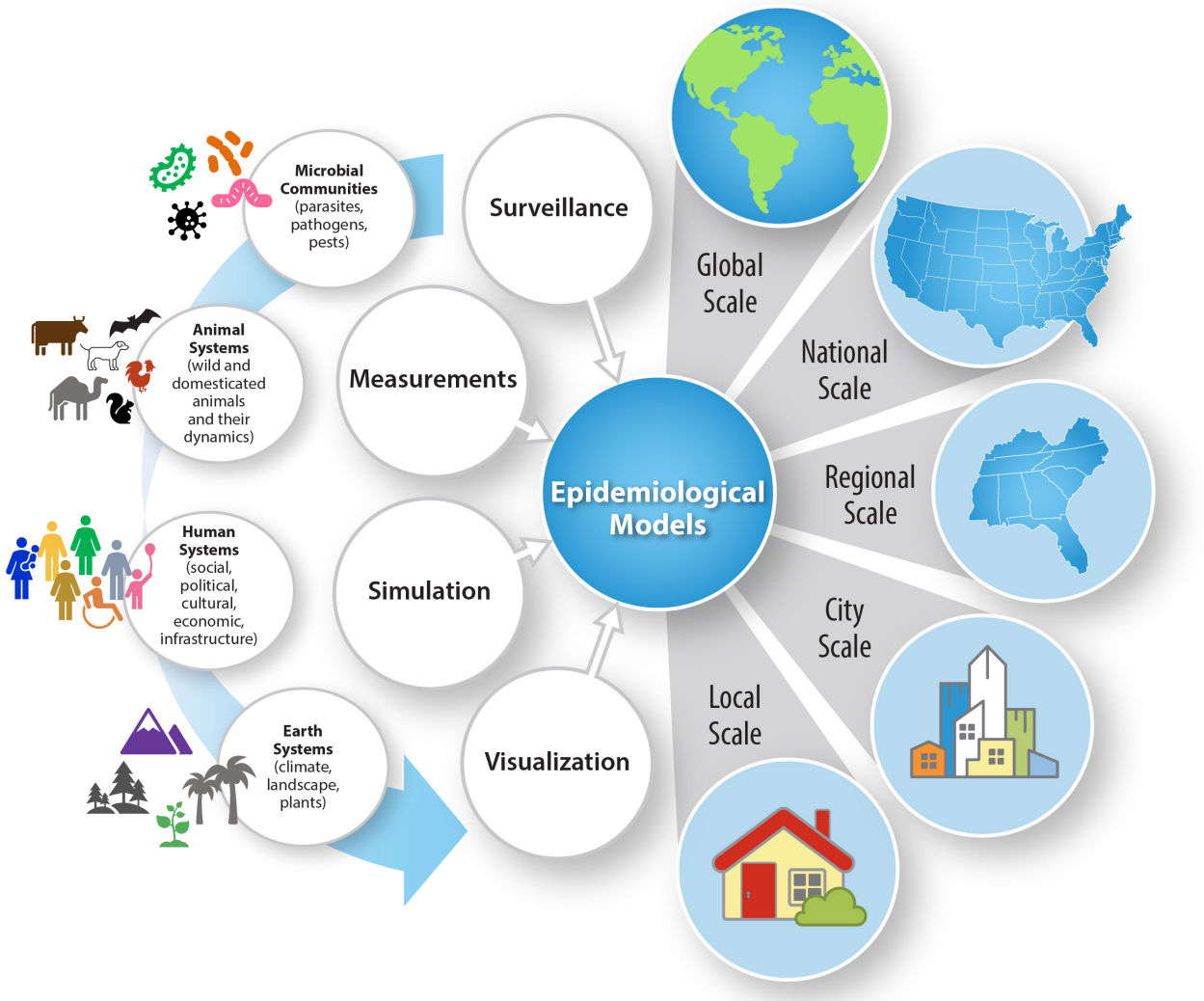

Fig. 4.1. Integrated models that represent the interrelationships and behavioral responses of the four key ecosystem components across space, time, and disciplines are necessary to accurately represent and quantify disease impacts.

4.2 Priority Research Opportunity

Goal: Elucidate Multiscale Ecosystem Complexities for Robust Epidemiological Modeling

Accurate representations of human-environment interconnections, particularly among the four key ecosystem components (i.e., human, animal, microbial, and Earth systems), are necessary to correctly model and quantify disease impact (see Fig. 4.1, this page). Traditionally, epidemiological models have focused only on modeling disease dynamics within individual ecosystem components. Addressing this gap requires not only integrating validated models across space, time, and disciplines, but also assimilating real-time heterogeneous data streams that capture behavioral responses to environmental changes and interventions. Flexible, scalable, and disease-agnostic modeling frameworks will dramatically improve the nation's ability to prepare for and quickly respond to emerging biological threats. Creating multiscale ecosystem approaches that leverage DOE computational facilities

Common Epidemiological Modeling Terminology

Since the 1850s, mathematical and statistical models for infectious diseases have been instrumental in providing critical understanding, informing mitigation, and eradicating diseases. Modern advancements in scientific foundations of epidemiological forecasting allow modeling capabilities to continue assisting decision-makers in reaching risk-informed responses by providing real-time, on-demand, scenario-based analyses. Below are four common terms in epidemiological modeling that can aid in decision support.

Multiresolution modeling captures different degrees of detail and important elements within the modeled system, such as time, space, individual versus population level, and species (e.g., humans, animals, plants, vectors).

Backcasting is the ability to successfully predict past epidemiological trends, such as the number of cases, deaths, hospitalizations, or infection rates, based on previously reported data.

Surrogate or reduced models are simplified versions of more complex models that successfully approximate true dynamics while reducing computational burden.

Many-objective robust decision-making is an iterative decision-making framework consisting of (1) eliciting stakeholder objectives, (2) generating alternative problem formulations, and (3) assessing potential trade-offs between different objectives. This framework is used to reach consensus on robust actions that meet goals over a wide range of plausible futures.

will help anticipate and reduce impacts to health, society, the environment, the economy, and infrastructure.

4.3 Scientific Impact

Accurate representation of human-environment interactions and responses, particularly among human, animal, microbial, and Earth systems, and development of multiscale ecosystem models would support a greater understanding of perturbations and changes in humans and the environment. Moreover, these advancements would ultimately assist in identifying disease drivers and quantifying their impact on emerging and re-emerging threats. Due to the complexity in integrating across scales, species, systems, components, and models, large-scale disease-agnostic computational frameworks capable of combining model ensembles are needed to build and experiment with epidemiological models (see sidebar, this page). This type of framework is also required to elicit meaningful differences in model projections under different scenarios.

Infectious disease epidemiology has a legacy of disease-specific model development that renders them less effective with emerging diseases. Moreover, current models are not easily deployable across various space and time scales. A flexible, scalable, disease-agnostic modeling framework will significantly improve our nation's preparedness and response capabilities for emerging biological threats. On-demand, production-ready data assimilation systems and models that ingest real-time data streams will be able to continuously adjust to changing signals across ecosystems. Furthermore, novel, multiscale data assimilation algorithms will combine uncertainties due to parameters, models, and data to mutually inform and produce robust estimates of on-the-ground current conditions.

Given that epidemiological forecasts are ultimately designed for public health administration, usability of model outcomes relies heavily on quantified uncertainty of forecasts to ensure a successful translation of science into practice. Computational frameworks for integrating epidemiological models and complex

Four Scientific Challenges

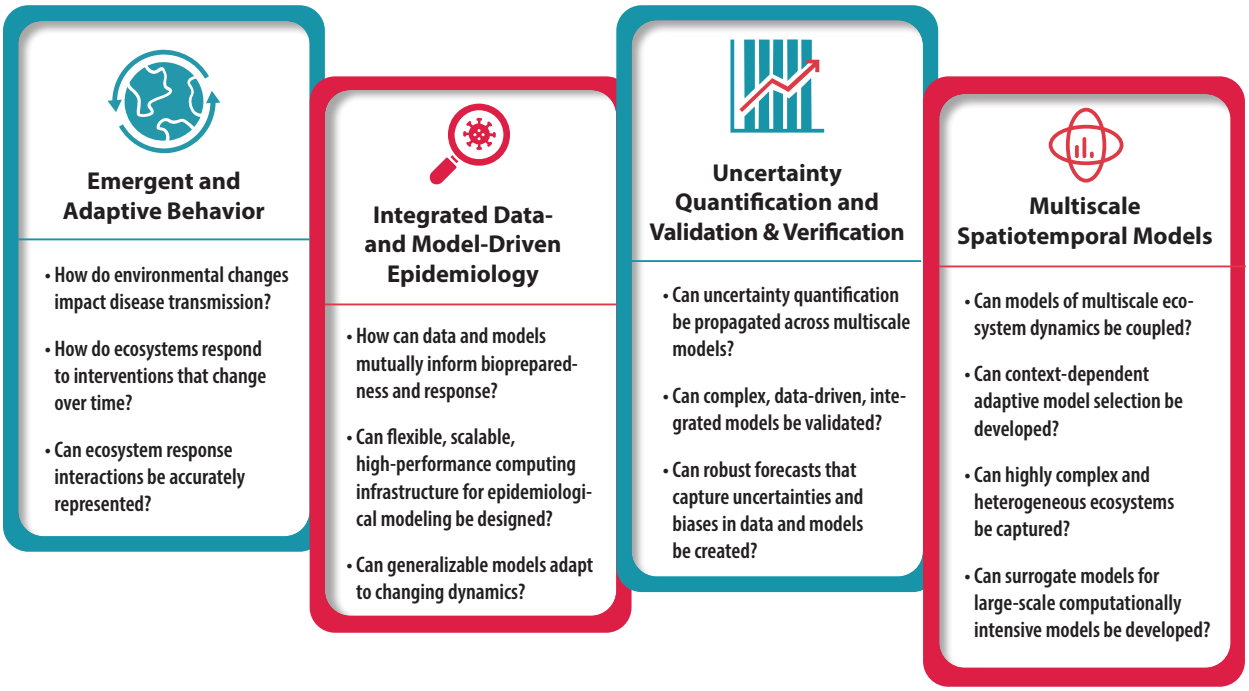

Fig. 4.2. These key scientific challenges outline an integrated, multiscale framework necessary to respond to future biological crises. Uniting these four core areas into an interconnective model highlights responses to environmental perturbations and provides greater insight into the interactions and outcomes of the entire global ecosystem.

analyses on HPC resources need to support uncertainty quantification. Such frameworks also need to support verification and validation analyses, including multiresolution model docking, backcasting, model reduction, and surrogate model training, among other applications. Leveraging HPC resources available across the DOE complex, combined with large-scale probabilistic sensitivity analyses, such as many-objective robust decision-making approaches (Kasprzyk et al. 2013), will enable intervention strategies that perform well across multiple goals, future uncertain scenarios, and models.

4.4 Scientific Challenges

This priority research objective focuses on multiscale ecosystem complexities for robust epidemiological modeling. To achieve this goal, four major scientific challenges need to be addressed: (1) enabling models to incorporate emergent and adaptive behavioral response to environmental changes and interventions, (2) developing new approaches that combine data and models to accurately describe complex relationships, (3) quantifying uncertainty across data and models and validating and verifying newly developed multiscale spatiotemporal epidemiological models, and (4) linking or coupling multiple scales over time in epidemiological models to understand the impact of spatiotemporal interactions on the overall disease system (see Fig. 4.2, this page).

Disease occurs when there is alignment of the host system, pathogen stages, and environmental conditions. However, most epidemiological models have primarily focused on human factors, sometimes incorporating the environment, and are implemented after the detection of human disease. Applying an integrative, multiscale ecosystem approach

that harnesses the interconnectedness of microbial communities, including pathogens, parasites, and pests, along with Earth and environmental systems, animal systems, and human systems, will provide a completely new and holistic way of viewing disease. This approach enables advancements in epidemiological models through more accurate representations of how, why, when, and where a disease may occur. Additionally, these models enable the quantification of intervention strategies across the different systems and the feedback loops within the systems. Novel approaches are needed that couple human, animal, and environmental models at multiple scales and are disease-agnostic. However, due to the complexities of these models and interactions, new model reduction approaches that tease out the important drivers contributing to disease spread are needed as well as the ability to choose different components within the system based on the diseases or question of interest.

Scientific Challenge 1: Incorporate Emergent and Adaptive Behavior into Epidemiological Modeling Approaches

While computational advancements have enabled the use of large-scale data streams and sophisticated modeling approaches, fundamental gaps in epidemiological modeling capabilities persist. More specifically, existing models lack the ability to capture emergent and dynamic human behavior in response to new threats and evolving conditions. For example, the most common approach used to study epidemics (e.g., SIR-type models) ignores or oversimplifies heterogeneous mixing and behavioral responses, leading to potential erroneous outcomes. These behavioral responses drive interactions and play a fundamental role in disease transmission. The COVID-19 pandemic demonstrated the importance of understanding heterogeneous emergent behavior and the power that disinformation campaigns have in affecting the course of a pandemic. Developing mathematical and computational approaches that not only assimilate real-time behavioral data streams from across multiple scales and species but also adapt as conditions change will enable more accurate understanding of disease spread. Unfortunately, data governing behavioral responses to environmental changes and interventions (e.g., from public health departments, detailed surveys, GPS, cellular devices, and sensor data streams) are heterogeneous, changing, incomplete, and biased. These features present significant challenges for use in epidemiological modeling and require focused efforts to harmonize, curate, and manage. Thus, development and analyses of proxy data that can inform emergent and dynamic behavior are needed. Additionally, incorporating adaptive human, animal, and environmental behavior enables targeted and more effective responses in the presence of uncertainties and complexities within the system.

Scientific Challenge 2: Improve Forecasting Accuracy with Integrated Data- and Model-Driven Epidemiology

IBM estimates that we are generating 2.5 quintillion bytes of data each day, so much that 90% of the world's data was created in the last few years (Marr 2018). Additionally, the COVID-19 pandemic became a new driving force behind the data revolution, providing access to unprecedented data streams to understand, mitigate, and respond to this public-health crisis (CCDC 2021). Although machine learning (ML) and artificial intelligence (AI) approaches have been developed to extract features from these data streams, many fundamental processes cannot be recovered or understood through data analytics alone. New approaches are needed that combine data and models to accurately describe the complex relationships, impact of local and global effects, and overall disease dynamics. However, limited resources pose a problem for expanding data that is sparse, sensitive, and expensive to collect. Thus, researchers need to develop principled methods for understanding and surveilling sensing modalities to improve forecasting accuracy from integrated data and model-driven epidemiology.

Additionally, current epidemiological models require calibration, which is often a tedious step, to incorporate the elements of disease spread rate and disease presentation in humans (Eichner and Dietz 2003). The development of calibration methodology and automation of calibration workflows, interoperable with DOE leadership computing systems, will be essential to ensure

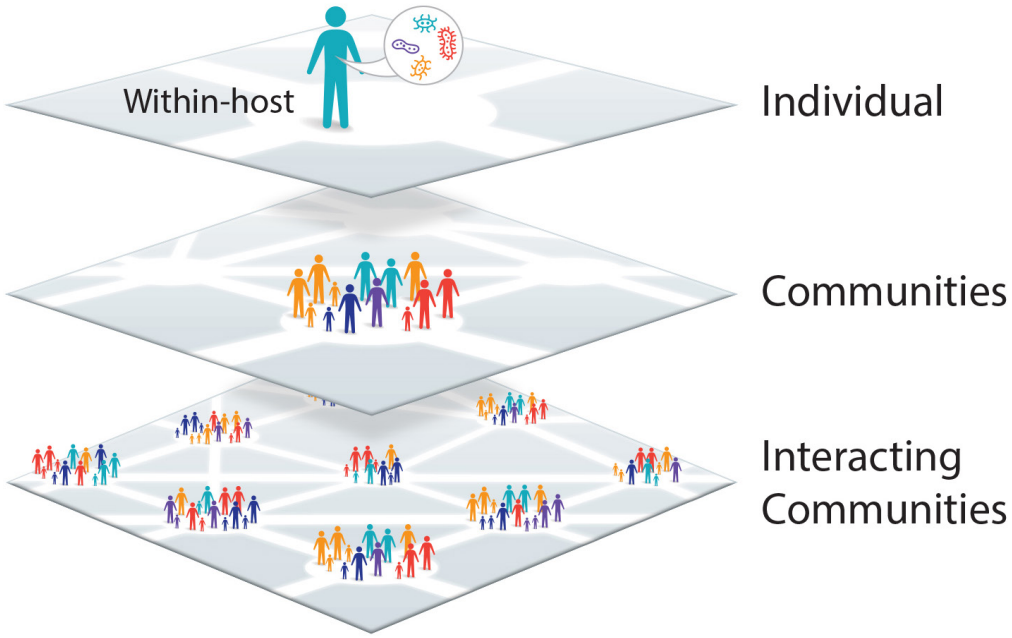

Fig. 4.3. To more accurately understand, model, and forecast diseases, epidemiological models need to capture the behavior of different viruses and bacteria in an individual as well as the heterogeneity in disease spread across individuals and populations. SEIR-type models do not consider individuals and chance events but rather uniformly connected populations in which everyone has the same probability of becoming infected; consequently, they are unable to capture heterogeneity. In contrast, agent-based models capture individual differences and chance events and thus more closely represent how disease spreads in the real world.

models integrate distributed and diverse datasets to help enable responsive decision support.

Scientific Challenge 3: Enable Uncertainty Quantification and Validation & Verification of Epidemiological Models

Despite the unprecedented production (Else 2020; Cai et al. 2021) and coproduction of scientific work (Ray et al. 2020; Borchering et al. 2021), individual research groups have generally worked independently to provide epidemiological model outputs. At times, these outputs have been combined to produce short-term ensemble forecasts of cases, resource needs, and disease outcomes. Nonetheless, the shortcomings in this partitioned approach are numerous, including the large amount of overlapping work that occurs within individual research groups seeking to exploit advances in HPC, data management, ML, and AI methods when developing, modifying, verifying, and validating epidemiological models. Moreover, this fractured modeling approach limits consensus around how best to incorporate, quantify, and represent major sources of uncertainty across different estimation approaches. As multiscale spatiotemporal models are developed, a clear understanding of key sources of variation across different aspects of the disease system and new algorithms for incorporating these sources into estimation procedures are necessary. Without explicit treatment, models may propagate and compound uncertainty throughout the system, leading to biased or false forecasts, predictions, and decisions.

Scientific Challenge 4: Develop Multiscale Spatiotemporal Models

The dynamics of infectious disease systems are inherently multiscale. Pathogens jump boundaries between animals and humans, replicate within hosts, and spread among host populations (see Fig. 4.3, this page; Garabed et al. 2020). While methods for calibrating models at specific scales exist and are relatively robust, model calibration in a multiscale, multifidelity setting remains an active research field. A fundamental scientific

challenge is how to link or couple multiple scales over time to understand the impact of spatiotemporal interactions on the overall disease system. In this context, the high-dimensional set of parameters presents a challenge, and the range of spatiotemporal scales at which these parameters interact requires a new set of model reduction algorithms that are challenged by the presence of nonstationary model dynamics. These models are informed by and benefit from a combination of scientific tools spanning biology, mathematics, statistics, and computational science.

Additionally, research groups often focus on a single modeling scope, with one modeling method (e.g., compartmental, meta-population, agent-based), geographical extent (e.g., city, county, state, national), and temporal scale (e.g., short-term forecast, medium-, or long-term planning). Even when multiple scopes are considered, they are rarely integrated into multifidelity ensembles that can mutually inform each other and be combined to support rapid decision-making during different stages of an unfolding public health emergency.

Examples of Basic Research Directions to Address These Challenges

Achieving an integrative, multiscale ecosystem approach requires successfully integrating data and models from multiple stakeholders, across disciplines (e.g., Earth systems, environment, animals, humans, pathogens, pests, and parasites) and scales (e.g., genome to ecological). Humans, animals, plants, pests, and pathogens are constantly adapting to external and internal influences, from small perturbations to large environmental changes. These changes may result in new and emergent behaviors or physiological shifts that can ultimately affect a range of scales, from the individual to ecosystem level.

Additional gaps require developing a family of epidemiological models that are sufficiently flexible to accommodate emergent and adaptive behavior of humans and animals during outbreaks as well as scalable data assimilation methods. These methods incorporate rich datasets—mostly of observable human activity—into disease models to estimate how behavioral changes impact diseases and their presentation in human societies, while also incorporating the effects of pathogen mutation over time.

Also needed is research into scalable calibration methods for models that are stochastic by construction (e.g., agent-based) and especially those that do not rely on some version of pseudo-marginalization (Blonigan et al. 2021). In addition, due to the difficulties of modeling human mixing in stratified populations, most disease models will be approximate. As a result, large model ensembles and super-ensembles (Yamana et al. 2016) will be required to mitigate model-form uncertainties. Large ensembles require a large quantity of data to model-average, which intrinsically will not be available for novel diseases. Thus, research is needed into developing parsimonious models that are commensurate with the amount of information present in available data. Finally, since much environmental and epidemiological data are limited, research into inference methods that fill in missing-at-random data is also required.

Approaches using detailed multiscale models in combination with ML techniques will create optimal measurements and proxy data streams for sparse, sensitive, or expensive-to-collect data sources. Efficiently combining data streams with multiscale ensemble models on large-scale computing resources requires new algorithms and enabling technologies.

DOE has a long-term investment, through the QUEST and FASTMath projects, in uncertainty quantification algorithms focused on multiscale, multifidelity model development. These are Scientific Discovery through Advanced Computing (SciDAC) institutes funded by DOE's Advanced Scientific Computing Research program. These developments focused on large-scale computational models for modeling turbulence, combustion, and Earth systems. In the context of epidemiological models, new algorithm developments are required to achieve reasonable time-to-solution for robust forecasts. Such developments include (1) sensitivity analysis and model comparison techniques for determining the appropriate model parsimony at each representative scale, (2) efficient sampling methods to evaluate disease dynamics scenarios, and (3) frameworks for coupling models across multiple scales, including transferring information between models with quantified uncertainty (Gorodetsky et al. 2020). Some epidemiological models (e.g., compartmental

models) can run with limited computational resources; however, running model ensembles jointly quickly becomes prohibitive, requiring the development of algorithms for surrogate (or meta-) models. These surrogates (e.g., Gaussian processes or Neural Networks) operate in high-dimensional settings and must often be built with limited information. The surrogates would replace individual models or model ensembles that span multiple scales and reduce the time-to-solution in epidemiological studies.

Uncertainties in parameter estimates for epidemiological models come from uncertainty in model structure, measurement noise and bias in empirical data, and embedded randomness in stochastic models. Traditional validation, calibration, and UQ techniques must expand to include human, animal, and climate interactions to incorporate the multiscale, multimodel, and multidata characteristics of the ecosystem modeling approach. Many computational toolkits systematically compare and validate simulation outputs against observed data and quantify how numerical and physical parameter variations affect simulation outcomes. Examples include Sandia National Laboratories' DAKOTA project (Adams et al. 2020), the Verified Exascale Computing for Multiscale Applications toolkit (VECMAtk; Groen et al. 2021), and the Extreme-scale Model Exploration with Swift (EMEWS) framework (Ozik et al. 2016). UQ methodology was traditionally designed for deterministic models, and key challenges remain with applications for complex stochastic models, both in terms of applicable statistical and mathematical algorithms as well as computational complexity.

Capabilities Required to Address These Challenges

Providing the underlying support necessary to integrate epidemiological surveillance models requires new methods that harmonize, curate, and validate the reliability of modeling workflow and supply access to novel data streams. Computational frameworks are required that support access to HPC resources available at DOE Office of Science user facilities. Such frameworks are necessary to run complex analyses and efficiently verify and validate models, such as multiresolution model docking, backcasting, model reduction, and surrogate model training, among other applications.

The scientific challenges described above outline the necessary advancements to drive the next generation of modeling—one that will bring current data together with historical insights and forecast potential outcomes to guide real-time decision-making. The advancements to foundational knowledge in epidemiological modeling discussed in this chapter will make possible a future where complex interactions and processes contributing to disease spread could be accurately modeled and, in this way, capture global ecosystem interactions, behaviors, and adaptations across multiple scales. DOE is well positioned to unify these key capabilities, thereby accelerating the research necessary to achieve this powerful new epidemiological modeling framework that would transform our nation's pandemic preparedness and support future decision-makers in responding to real-time impacts of biological crises on a global scale.

Chapter 5

Materials and Manufacturing

5.1 Introduction

In any biological event the first priority is to protect human life. To safeguard humanity during the COVID-19 pandemic, certain protocols were put in place, such as masking, social distancing, and washing or sanitizing hands. At the beginning of the crisis, uncertainties emerged as to which masks would be effective against the virus, whether the virus could survive on surfaces, and whether transmission primarily took place through the air or through surface contact. Answering these questions required understanding how the virus interacted with other surfaces, such as mask fabric, human skin, or common items encountered in daily life (e.g., shopping cart handles). Indeed, the molecular details of pathogen-material interfaces are critical to understanding how biological threats persist in and transmit through the environment.

Unfortunately, the pathogen-surface interaction is complex and difficult to characterize. As a result, we lack a full understanding of its complexity, and this knowledge gap has limited theoretical approaches. Empirical studies indicate that electrostatic interactions govern most adsorption events, followed by van der Waals forces (Kimkes et al. 2020; Zhang et al. 2021; Aydogdu et al. 2021). Exposed proteins and carbohydrates of pathogen surfaces vary widely. Their interactions with materials also depend on the degree of hydration, which only adds to the complexity. For example, viruses or bacteria in respiratory microdroplets interact with surfaces differently than virions or spores do as aerosols. This heterogeneity of pathogen-surface chemistry has not only led to case-by-case determination of personal protective equipment (PPE) effectiveness but also resulted in our inability to engineer materials for specific threats (Hill et al. 2020; Zhou et al. 2020; Zangmeister et al. 2020; see also, Appendix D: Technology Status Document—Foundational Science for Pandemic Preparedness).

Another impediment to characterizing pathogen-surface interaction is the difficulty of studying biotic-abiotic surfaces in their native environment. State-of-the-art methods with atomic or molecular resolution—necessary approaches for observing nanoscale viruses—mostly operate under vacuum conditions, in which a pathogen's structure may not be preserved. Limitations of cryo-electron microscopy (cryoEM), optical bioimaging methods, atomic force microscopy, and multiphoton near-infrared excitation techniques prevent these technologies from adequately addressing buried interfaces and ambient conditions across the different time scales in which a pathogen will attach to a surface and migrate.

However, achieving a detailed understanding of pathogen-surface interaction would yield immense rewards, such as:

- Manufacturing materials to control the adsorption, migration, and diffusion of pathogens on surfaces and within materials.
- Designing materials with antiviral and antimicrobial properties that are safe and circular at end-of-life to minimize waste.
- Creating next-generation smart, wearable sensors to provide real-time pathogen detection.

A transformational shift in materials development may also be necessary to accelerate discovery through high-throughput screens in organ chips and digital twins of living systems enabled by artificial intelligence (AI). Finally, in anticipation that limited resources and supply chain issues will occur during a biological event, modular and distributed manufacturing will be critical to meeting our nation's pandemic preparedness and response needs.

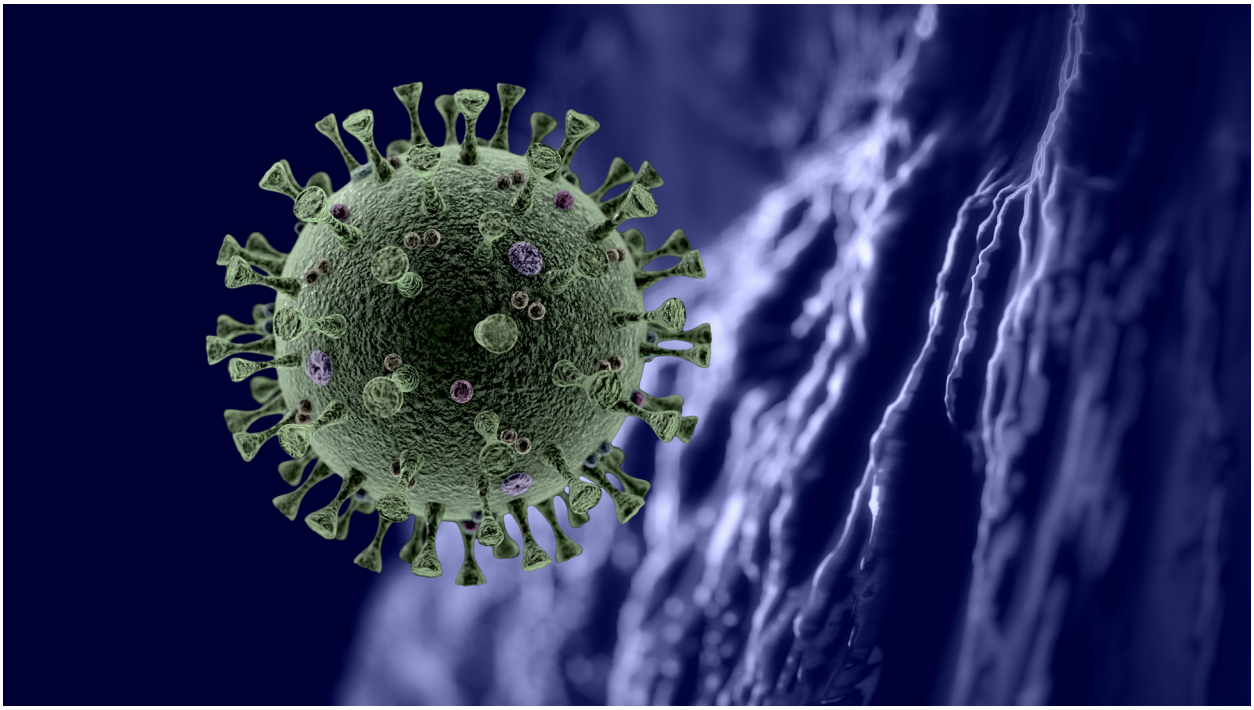

Fig. 5.1. The biotic–abiotic interface is key for materials and manufacturing biopreparedness.
[Image credit: Getty Images]

5.2 Priority Research Opportunity

Goal: Exploit Biotic–Abiotic Interfaces to Accelerate Design, Discovery, and Manufacturing of Materials for Biopreparedness

Being prepared for future biological events requires substantial fundamental research; this research must be fast-tracked to ensure the nation is fully equipped to mount a quick response. Our development of COVID-19 vaccines during the current pandemic serves as a prime example of this accelerated research need. Messenger RNA (mRNA) and liposomes were both discovered in the 1960s. Three decades later, the pair were tested as a vaccine for the flu virus in mice (Wolff et al. 1990). Two more decades passed before mRNA became a promising therapeutic tool for vaccine development (Dolgin 2021). When COVID-19 emerged in 2019, scientists were able to produce a vaccine in record time, based on this foundational research that began nearly 60 years ago.

A better understanding of biotic–abiotic interfaces—at surfaces and buried interfaces, in ambient conditions, and across time scales—is essential for the nation's ability to prepare for and respond to the next biological event. Therefore, to avoid a situation like the one described above and drastically reduce response time, the capabilities must be developed now for characterization and modeling. Only then can researchers exploit these interfaces to design and manufacture materials to meet future needs (see Fig. 5.1, this page).

5.3 Scientific Impact

Develop a Fundamental Understanding of Pathogen Interactions with Materials

Pathogens can remain viable on surfaces long enough to permit transmission. While usable on some surfaces, disinfectants may not be practical or compatible with certain materials. For example, spraying disinfectants on face masks can result in inhalation hazards, and autoclave sterilization degrades the filtration properties of N95 masks. However, if materials could be

endowed with antipathogen properties through their architectures and interfaces, touch-transfer modes of transmission would be minimized or possibly eliminated. Augmenting materials with antipathogen properties can also reduce the need for potentially performance-degrading sterilization techniques and prolong lifetimes, thereby reducing pressures on supply chains. Basic research could also address outstanding challenges in materials regeneration, reuse, and recycling after prolonged exposure to pathogens. Furthermore, existing materials with antipathogen properties may not be suitable for pathogens in future biological events, creating a need for pipelines to develop new materials. Acceleration of such advances will require a collective effort across industry, academia, and national laboratories, with particular emphasis on capabilities at DOE Office of Science user facilities.

Create Smart, Wearable Sensors for Emerging Pathogens and Tracking Mechanisms of Transmission

Advanced technologies capable of detecting pathogen presence, identity, and activity could form the basis for smart sensors that could be worn or placed as sentinels in public spaces. Scientific challenges underlying development of such sensors include (1) understanding binding mechanisms between pathogens and abiotic substrates; (2) designing, synthesizing, and stabilizing sensitive and selective substrates; and (3) creating and understanding signaling mechanisms (optical, electrical). Electrochemical reactions can provide one potential transduction approach (Bobrinetskiy et al. 2021). Research directed toward nontraditional sensing modes, such as quantum-based sensors, offers the promise of not only identifying pathogen presence, but also quantifying viral load (Li et al. 2021). Research efforts could include exploring new plasmonic, magnetic, or electroactive 2D and colloidal nanomaterials functionalized to interact with pathogens at their interfaces (Altug et al. 2022). Practical outcomes of such basic research will accrue at two levels: the individual (e.g., exposure measure and intervention) and epidemiological (e.g., rapid contact tracing and early warning). In parallel, these efforts will advance our fundamental understanding of interactions (e.g., electronic, steric, and chemical) between biological moieties and inorganic, organic, or polymeric substrates.

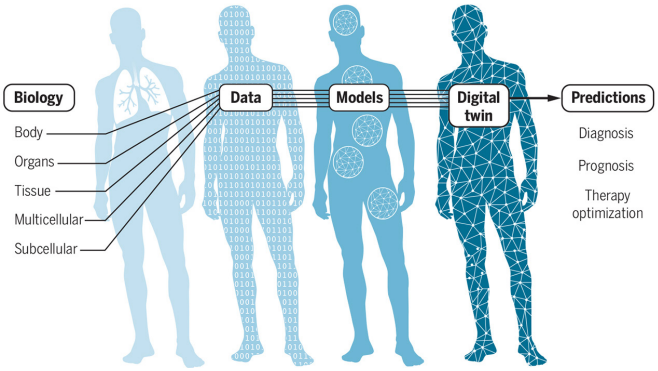

Fig. 5.2. The biotic-abiotic interface is extremely difficult to probe with *in vivo* experiments. Thus, data-driven validated digital twins will be invaluable tools to predict behavior under varying conditions.

[Image credit: Reprinted with permission from AAAS from Laubenbacher, R., et al. 2021. "Using Digital Twins in Viral Infection," *Science* **371**(6345), 1105–106. DOI:10.1126/science.abf3370]

Elucidate Design Rules for Bioactive and Biohybrid Materials with Programmable Bioresponse

One approach for achieving this priority research objective may include replicating the biological systems features in synthetic models, which will allow us to experiment on these models to reveal the underlying mechanisms of pathogenesis and predict the most appropriate means of therapeutic administration. Recent progress in additive manufacturing (Mota et al. 2020; Bernal et al. 2019; Colosi et al. 2016; Liu et al. 2017) has created an opportunity to develop agile biological makerspaces that will catalyze new ways for manufacturing living matter with unprecedented resolution, complexity, and scale. Hierarchies of artificial and biological materials with living cells can serve as mimics of natural tissues and organs (Murphy and Atala 2014; Hinton et al. 2015; Place et al. 2009; Lutolf and Hubbell 2005). Integrating these organoids with fluidics can yield organ chips that reproduce the bioresponse to pathogens, vaccines, and therapeutics. These reproductions could make it possible to validate multiscale models for that bioresponse to the point that it becomes predictable (e.g., in a digital twin). If realized, AI-enabled digital twins could lead to a transformational shift in materials development for biopreparedness (see Fig. 5.2, this page).

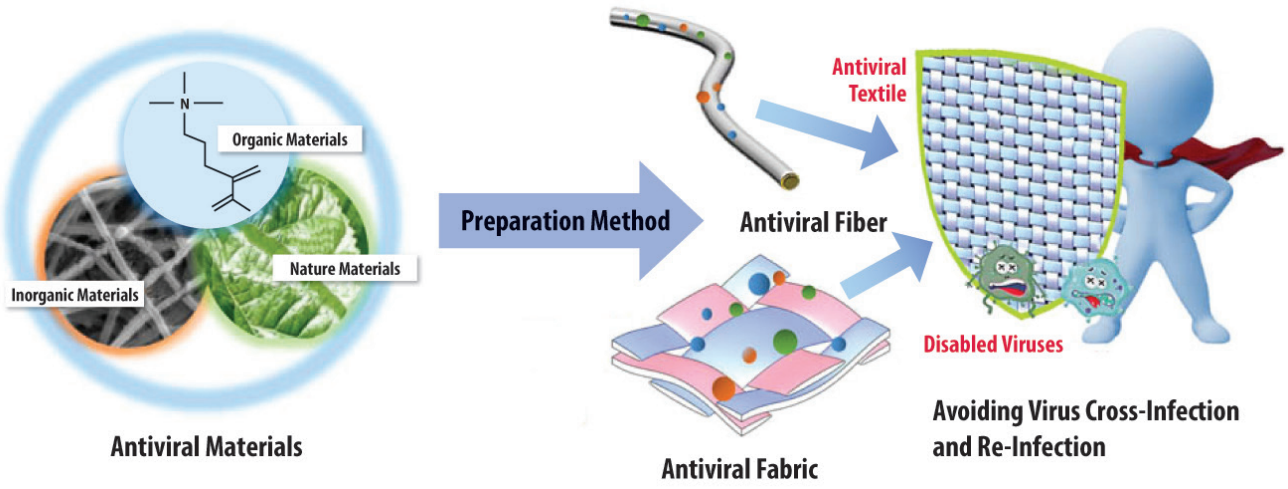

Fig. 5.3. Functional organic and inorganic materials can be used to control pathogen interactions, such as those occurring in the porous materials used to manufacture personal protective equipment.

[Image credit: Reprinted under Creative Com mons 4.0 International License from Zhang, Y., et al. 2021. "Application of Antiviral Materials in Textiles: A Review," *Nanotechnology Reviews* **10**(1), 1092–115. DOI: 10.1515/ntrev-2021-0072.]

5.4 Scientific Challenges

Scientific Challenge 1: Control the Binding, Migration, Diffusion, and Neutralization of Pathogens on and Within Materials for Protection, Safety, and Circularity at End-of-Product-Life

Existing materials with antiviral and anti-microbial properties may not be suitable for dealing with pathogens in a future biological event. As a result, pipelines are needed to develop new materials (Rakowska et al. 2021; Meselson 2020; Firquet et al. 2015; Vasickova et al. 2010; Chin et al. 2020; Sizun et al. 2000; Xue et al. 2020; Joonaki et al. 2020). Certain polymers—such as metallic nanoparticles along with photo-oxidizing organic and inorganic materials—have exhibited antiviral and antimicrobial properties. As such, they can be used as coatings on surfaces or manufactured as part of a diverse array of protective materials (see Fig. 5.3, this page). However, we have a limited understanding of pathogen interaction with materials and of the materials' safety level. We also lack an understanding of how adding antipathogen features affects the material's primary purpose. For example, the affinity of pathogens to surfaces will be dictated by the installed chemistries, which, in turn, can affect a pathogen's migration rate along surfaces. At longer time scales, pathogen diffusion in porous materials may affect the lifetime of the material. Furthermore, if surfaces are designed to neutralize the pathogen, it is unclear how the cumulative effects would impact performance under different exposure scenarios (Luan et al. 2018; Poon et al. 2020; Hizal et al. 2015).

A key scientific challenge is understanding how the integration of antipathogen features into materials affects their lifetime. Addressing this challenge will require identifying circumstances (e.g., extreme temperature or humidity) that render them ineffective. Material properties may diminish in efficacy over time (e.g., due to fouling, shedding, photobleaching, or corrosion). Thus, understanding mechanisms for material regeneration may also be important. While the ideal scenario in future pandemics would involve only a single pathogen, ensuring effective biopreparedness may require designing materials protective against multiple classes of pathogens. Hierarchical approaches could be used to design and integrate different classes of organics, inorganics, polymers, and nanomaterials with common materials serving as a first-line defense against emerging pathogens, such as those used in manufacturing personal protective equipment (PPE). However, given the importance of materials in limiting transmission rates and the likelihood that materials will feature enhanced protective measures, hierarchical approaches

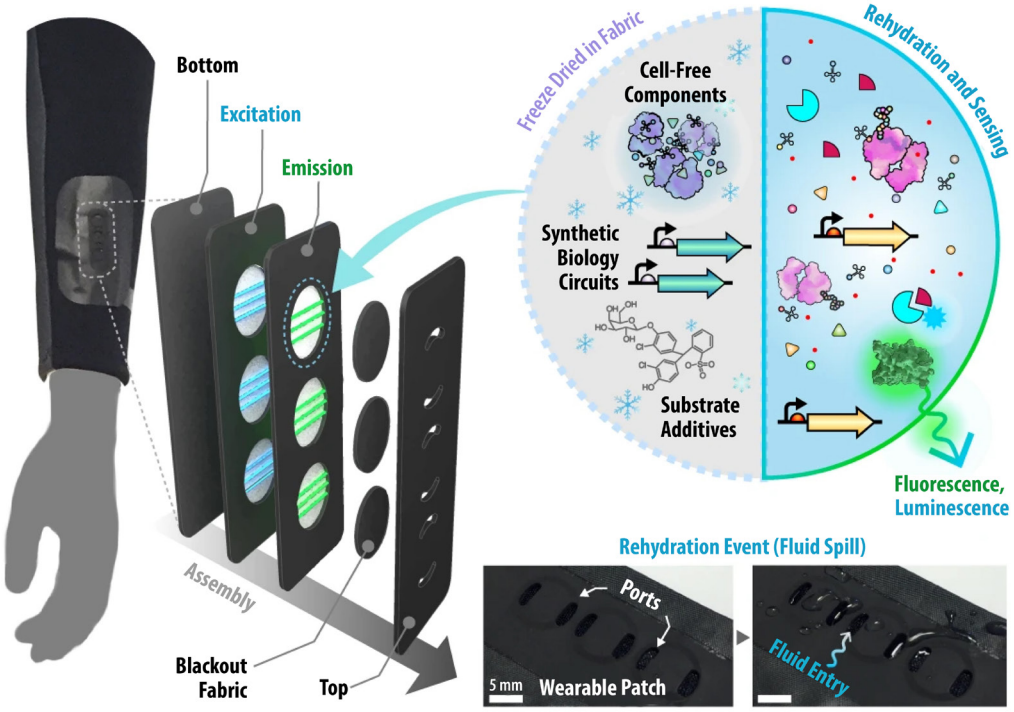

Fig. 5.4. Wearable biosensors integrated into textiles provide real-time detection, monitoring, and reporting capabilities for emerging pathogens.

[Image credit: Reprinted by permission of Springer Nature from Nguyen, P., et al. 2021. "Wearable Materials with Embedded Synthetic Biology Sensors for Biomolecule Detection," *Nature Biotechnology* **39**, 1366–374. DOI: 10.1038/s41587-021-00950-3.]

pose a significant challenge in managing waste. Strategies have emerged for deconstructing materials into their basic components, such that they may be remanufactured after reaching the end of their service life. Another challenge is to leverage advances in chemical recycling that can account for complex material hierarchies. Doing so would allow us to retrieve components providing protective benefits against pathogens to both prevent the discharge of hazardous materials into the environment and to reduce supply chain pressures.

These research directions also highlight the need to identify routes for manufacturing smart materials at scale to create less waste and alleviate strains on supply chains. An integrated approach for accelerating and scaling scientific breakthroughs from bench-scale research to development and beyond could involve the creation of digital workflows that combine advanced computation, autonomous experimentation, high-throughput characterization, and machine learning. Materials manufacturing could also be assisted by integrating *operando* characterization capabilities in manufacturing processes, thereby enabling adaptive control over processing parameters in real time.

Scientific Challenge 2: Design Responsive and Resilient Materials for Detection and Protection Through Characterization and Understanding of Biotic–Abiotic Interfaces Under Realistic Conditions

Pathogen detection and monitoring are critical to biopreparedness. Sensors typically exploit pathogen-binding biomolecules to provide selectivity through affinity. However, the presentation and binding ability of these biomolecules at sensor surfaces are problematic due to heterogeneity, pervasive fouling, and limited schemes for amplifying signal above background. Consequently, many biosensors suffer from false-negative results, long response times, or poor sensitivity. Thus, a persistent challenge is designing materials for detection (biosensors) and protection

(wearables and implantables) from the perspective of biotic-abiotic interfaces (see Fig 5.4, p. 26).

Pathogen size and structural complexity present a scale-bridging challenge for understanding and controlling molecular and nanoscale interactions with materials. Spatial and temporal dimensions are both relevant, with the former spanning nanometer to millimeter scales and the latter from sub-picosecond to at least 10^2 seconds (Schleicher et al. 2017). Approaches include atomistic (e.g., density functional theory) and classical molecular dynamics (MD) to understand structure, dynamics, and evolution of biotic-abiotic interfaces (Brancolini and Tozzini 2018; Subbotina and Lobaskin 2022). Innovative research toward accurate coarse-graining algorithms, enhanced by machine learning, may be necessary, as the chemical and structural descriptions of both pathogen and surface incorporate molecular, chemical, and topological features. Model validation with spectroscopic, structural, and morphological probes will be essential. Research challenges include enhancing the spatial and temporal resolution in *operando* studies under relevant conditions to scales accessible to atomistic or coarse-grained simulations. Multiscale cooperative behavior at the interface stems from hierarchical interactions—electrostatic forces, solvation, vibrational degrees of freedom—that are poorly understood and require innovations in both models and characterization (e.g., X-ray footprinting) to serve as guides for materials design.

An understanding of pathogen-material interfaces under realistic conditions could enable researchers to tailor and sustain the mechanical, electrical, electrochemical, and optical response of sensor materials used in diagnostics (Talebian et al. 2020; Nguyen et al. 2021; Ates et al. 2021; Kevadiya et al. 2021; Heikenfeld et al. 2019; Shrivastava et al. 2020; Yesilkoy et al. 2019; Neubrech et al. 2017; Rodrigo et al. 2015; Lopez et al. 2017; Squires et al. 2008). To sustain sensing ability, sensor surfaces must remain available for binding to molecules and pathogens. Traditional methods to reduce nonspecific binding rely on protein-based blockers, detergents, or hydrophilic polymers. However, these confer limited resistance in real-world samples comprising complex biological media. For many sensors, particularly those comprising nanomaterials and their arrays, their "hot spots" have nanoscale dimensions that can pose challenges for biomolecular and pathogen detection exclusively from affinity. Mechanisms for amplifying the signal above background are also needed to produce a selective and sensitive response. However, such mechanisms are often lacking. Genetically encoded sensors based on toehold switches, transcriptional factors, riboswitches, fluorescent aptamers, or clustered regularly interspaced short palindromic repeats (CRISPR) complexes may play a role in future biosensors, enabling both bench-top diagnostics and wearable sensors (Nguyen et al. 2021). Developing these capabilities may involve integrating biomolecular circuits or living cells (e.g., engineered bacteria) with functional nanomaterials in flexible substrates. This integration necessitates careful consideration of the biotic-abiotic interface, not only between nanomaterials and pathogens, but also between the sensor components. Developing scalable processes for defining both architectures and interfaces in biohybrid materials for sensors and wearables is also a challenge for biomolecularly precise manufacturing.

Scientific Challenge 3: Reveal Underlying Mechanisms of Biological Responses to Pathogens and Materials Using Bioinspired or Biohybrid Devices and Reproduce the Bioresponse with Machine Intelligence and Digital Twins

Organoids consisting of 3D cell hierarchies, differentiated from stem cells, can be integrated with different biological and artificial scaffolds and matrices to produce biohybrid materials for *in vitro* study of host-pathogen interactions (Kratochvil et al. 2019; Grigoryan et al. 2019; Hofer and Lutolf 2021; Brassard et al. 2021; Ingber 2022; Clevers 2016; Lancaster and Knoblich 2014; Laurent et al. 2017; Blatchley and Gerecht 2020). However, characterizing the immune response of different organs remains an outstanding challenge because such existing biohybrid materials lack immune cells. Furthermore, managing nutrient delivery over time can be difficult in the absence of a vascular network. Basic research along these lines could lead to breakthroughs for regulating self-organization of cells to generate organoids that develop deterministically into physiologically relevant shapes and sizes.

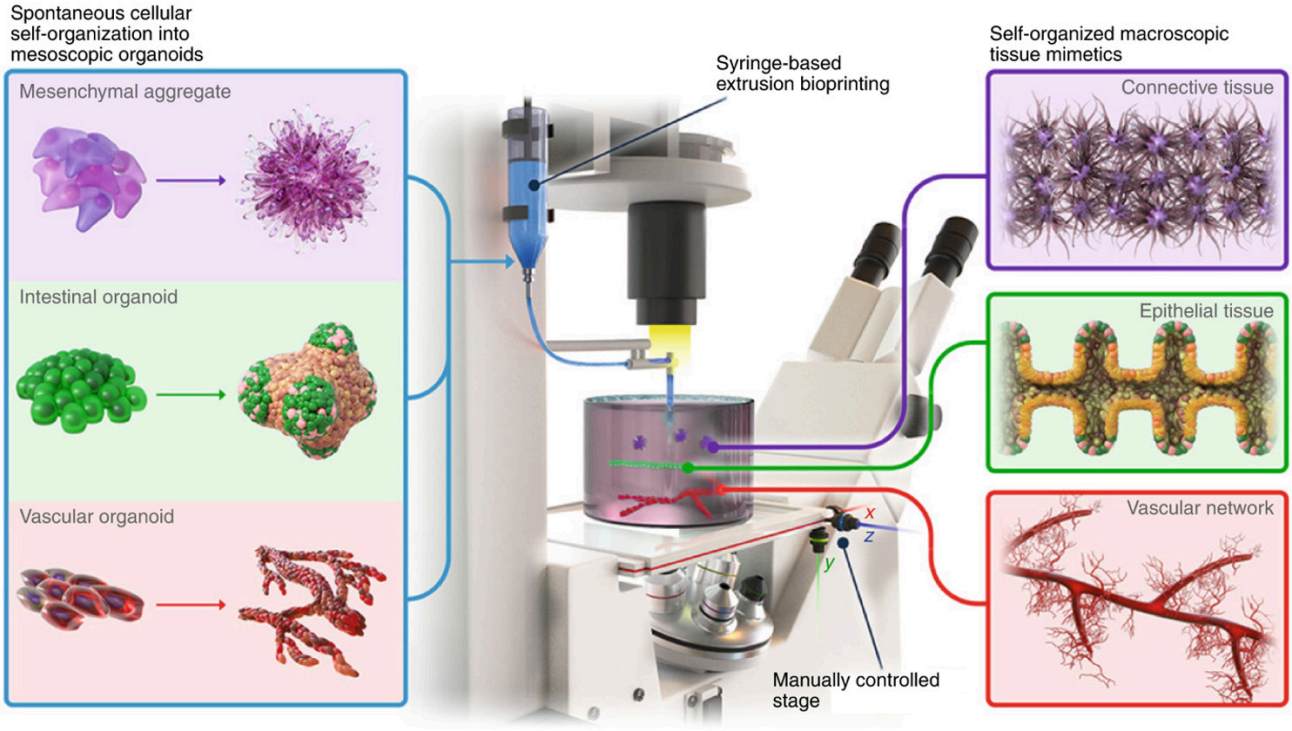

Fig. 5.5. Materials development exploiting biotic-abiotic interfaces can enable the creation of organoids for understanding the biological response to pathogens and therapies. In this example, syringe-based extrusion bioprinting is coupled to a microscope with a manually controlled stage to print bioinks composed of organoid-forming stem cells within matrices. The printed constructs are guided geometrically to self-organize into tissue-mimetic intestinal and vascular organoids with luminal structures.

[Image credit: Reprinted by permission of Springer Nature from Gartner, Z. J., et al. 2021. "Guiding Tissue-Scale Self-Organization," *Nature Materials* **20**, 2–3. DOI: 10.1038/s41563-020-00885-1.]

Developing this technology requires designing materials, particularly at the biotic-abiotic interface, to control the extracellular environment and direct organoid growth toward a desired architecture. Responsive and adaptive synthetic materials (i.e., beyond Matrigel) are also needed to provide time-dependent or spatiotemporally programmable mechanical and biochemical cues. To succeed, these pursuits also require the bioprinting of microstructured cell culture scaffolds with capabilities to direct stem cell differentiation by designing materials for controlled release of developmentally relevant molecules, locally or in gradients in space and in time (see Fig. 5.5, this page). Furthermore, it may also be possible to design bioprinting materials and processes to vascularize and prolong the lifespan of organoids to create mature, functional tissues that reach homeostasis.

By combining organoids with fluidics, we can control the microenvironment and enhance tissue function to characterize host-pathogen interactions under more realistic conditions. Research directions aiming to incorporate the immune system and blood vessel cells into organoids will be critical, potentially leading to organ chips that better emulate pathogen-induced responses. Enabling these research directions will require biosensors integrated into organ chips for real-time monitoring of cell behavior, environmental cues, and infection dynamics. Advanced 3D, 4D, and multimodal imaging capabilities harnessing photons, electrons, or neutrons are necessary to reveal the length and time scales of important events. Additionally, crosstalk between organs can be important in understanding the biological response to pathogens. As such, it will be useful to design interconnected fluidic

systems that comprise multiple organoids to model systemic responses of tissues and organs to infections. The long-term goal of this endeavor is to create "body-on-a-chip" capabilities that will accelerate knowledge building on pathogenesis, preclinical drug development, and innovative therapies.

Understanding and predicting bioresponse to materials and pathogens require developing and validating models of biological processes across scales (e.g., associated with internalization, replication, release, and the immune response). Synchronously collecting several types of measurements (physical, chemical, and biological) at different physiological scales could aid in model construction and validation. If realized, these advancements could lead to more accurate predictions of pathogen turnover rate, pathogen and infected cell lifespans, or pathogen production rates in infected cells. Whereas models in the past have been limited by space- or time-averaged assumptions, future models might seek to capture spatiotemporal heterogeneity in microenvironments, dynamics, and transport. Here, the challenge will be in the "big data" generated in advanced organoid studies. If successful, digital twins of living systems will allow more accurate predictions in the future for emerging pathogens and materials providing therapeutic benefit (Laubenbacher et al. 2021; Goyal et al. 2020; Sego et al. 2020). Understanding material-pathogen science is synergistic with the sensing, synthesis, and modeling challenges across the other research opportunities.

Capabilities Required to Address these Scientific Challenges

DOE's Office of Science user facilities are ideally suited for the nondestructive, multiscale, and *operando* interrogation of biotic-abiotic interfaces required for this work. Spectroscopic, scattering, and imaging methods can be used to characterize the structure and dynamics of the complex, hybrid pathogen-material interface. For example, extreme sensitivity and resolution allow for analyzing low concentrations down to the atomic scale or probing a structure's top atomic layer. Furthermore, the user facilities' world-leading experimental capabilities and expertise in high-performance computing resources using AI, MD simulations, and modeling will be critical as we analyze increasingly complex pathogen interactions and work to establish digital twins to enhance prediction and preparedness capabilities (see Fig. 5.2, p. 24).

Research carried out to address the scientific challenges above will provide the foundational knowledge necessary to transform our understanding of the biotic-abiotic interface. This understanding would, in turn, revolutionize the use of materials in biopreparedness, offering (1) pathogen-agnostic PPE that minimizes virus transmission and alleviates supply chain issues, (2) smart fabrics that protect wearers and alert them to the presence of a pathogen, (3) antiviral surface coatings that prevent transmission and neutralize the virus, and (4) next-generation sensors that supply cheap, scalable home tests equal in sensitivity to current laboratory-based tests.

Chapter 6

Crosscutting Themes

6.1 Introduction

The urgent need for sophisticated experimental capabilities, complex simulations, and data analysis cuts across all aspects of bioscience research and is necessary for improving biopreparedness for rapid response to future pandemics or biological events. Cutting-edge experimental tools for determining genomic sequence and molecular structure are the starting points for diagnostic and therapeutic development as well as for tracking the emergence and evolution of pathogen variants. High-throughput and high-precision measurements of molecular interactions are central to testing and validating the efficacy and safety of proposed therapeutics. A detailed understanding of interactions between proteins and membranes and between different protein complexes is necessary to disrupt virus transport and replication (Ludwig 2011; Hackstadt et al. 2021). High-performance simulation helps understanding of the structure of pathogen proteins and their function in human infection and disease. Large-scale data analysis identifies potential data-driven hypotheses and facilitates the search for patterns of evidence across global-scale populations. The complexity of life, environments, and biological systems leads directly to models that must incorporate large ranges of physical scales and complex networks of interactions in large, dynamic populations. Scales range from molecular to cellular systems to organisms and populations. Data driving these models are growing exponentially in scale and complexity and are globally distributed. DOE experimental and computational facilities are critical resources for this research and serve as test beds for developing new classes of bioscience methods and tools.

The roundtable's Crosscutting Themes panel considered this broad space of capability and needs in experimental systems, facilities, computing, and data. The focus on research opportunities is motivated by two specific gaps (see Fig. 6.1, this page):

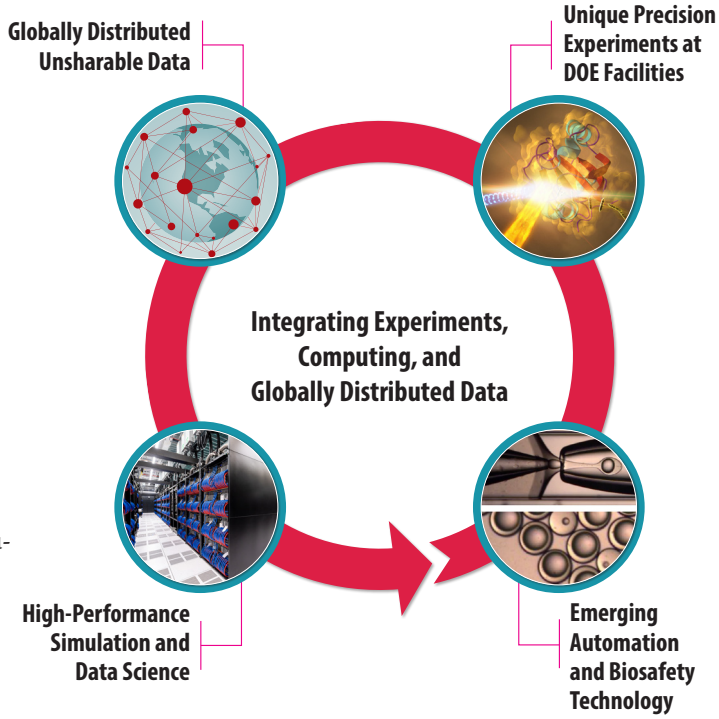

Fig. 6.1. Accelerating the iterative experiment-compute cycle and supporting access to globally shared and distributed data will significantly increase automation of the analytical process and result in more timely insights.

[Image credits: Clockwise from top left, Getty Images, SLAC National Accelerator Laboratory, Lawrence Livermore National Laboratory, Oak Ridge National Laboratory.]

1. Today, computational and experimental methods and processes are largely separate activities, integrated only by human-managed interactions. This gap critically slows the iterative experiment-compute cycle needed in complex workflows incorporating multidomain experiments and data, multiscale computational models, and active artificial intelligence (AI)-based analytics. The lack of integration limits our ability to rapidly produce data and models necessary

for responding to biological threats such as a pandemic.

2. Capabilities for data collection have grown tremendously, but further advancement is limited by the inability to bring together large, distributed datasets to support pattern search and model development. Biology is a global enterprise undertaken at multiple scales by both public and private organizations. In many experiments, data are limited due to inability to move or share data for either technical reasons—such as scale, complexity, or communication limitations—or policy reasons—such as privacy restrictions on medical data or intellectual property limits for molecular data. These limitations mean that patterns crossing these boundaries where data are not visible or shareable cannot be included and therefore will not be part of the resulting models.

6.2 Priority Research Opportunity

Goal: Accelerate Biopreparedness by Integrating Experimentation, Computing, and Globally Distributed Data

The innovative research needed to accelerate scientific discoveries for biopreparedness requires a new paradigm that integrates experimental, computational, and data techniques. A systems approach will enable researchers to combine complex heterogeneous data with autonomous experiments and real-time simulations. This approach would support efficient experiment-compute iterative processes and provide tools for data-to-knowledge transformations. Enabling scientific advances will also require new computational frameworks for model development, along with secure and privacy-preserving data and metadata access, curation, and quality management (see Fig. 6.2, this page). These foundational capabilities intersect with each of the priority research opportunities and, if realized, will accelerate breakthroughs in bioscience and biopreparedness.

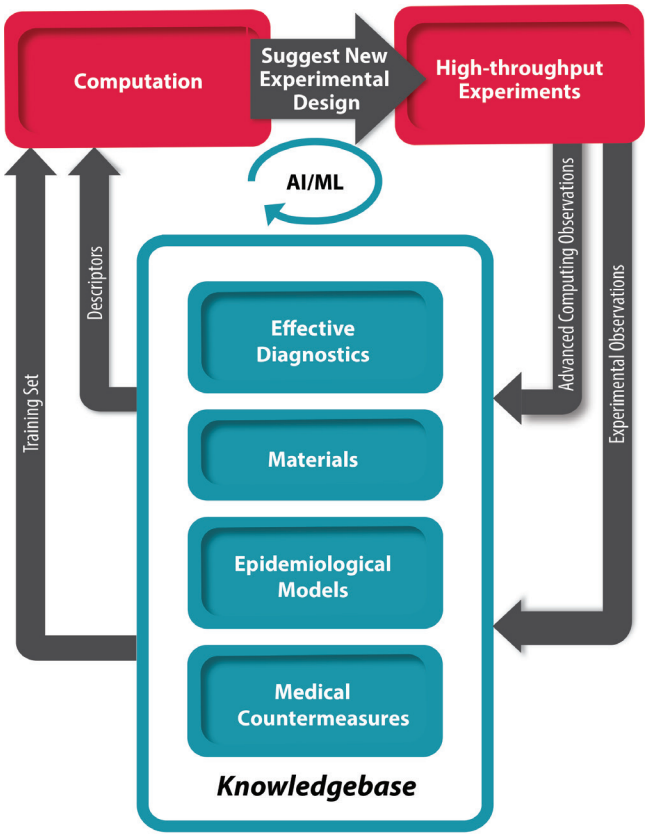

Fig. 6.2. New automated workflows that integrate the experiment-compute cycle will provide real-time insights from a distributed data ecosystem and create a unique national test bed for accelerating bioscience.

6.3 Scientific Impact

Fundamental to all research conducted in the context of biopreparedness and response is the need to efficiently integrate experimental, observational, and computational results. More importantly, creating a rapid feedback loop between these different capabilities will accelerate the response during future crises and, at the same time, enhance the quality of scientific results. By successfully bridging the fundamental gaps between data science and demanding experimental approaches, a paradigm shift in how research is conducted would occur with the potential to create greater understanding of these biological systems and the pathogens that threaten them. This paradigm shift will allow scientists to develop strategies enabled by AI-based autonomous experiments and simulations to gain greater insights, more efficiently guiding the exploration and validation of all available information spaces. These fundamental capabilities are essential for

creating extremely fast feedback loops between computation and experimentation that will enable rapid and intelligently designed experiments necessary for downstream development of effective diagnostics, epidemiological models, and countermeasures to address emerging biothreats.

Rapid response to future biothreats requires a complete structural, chemical, and dynamic description of both the threat and its targets. Current experimental techniques provide information for a single length scale, time scale, probe (e.g., electrons, light, or neutrons), phenomenon (e.g., X-ray diffraction or X-ray spectroscopy), or physical-chemical property (e.g., electrochemistry). Reliable and accurate characterization requires integrated multiscale and multimodal analysis. The combination of systems biology and synthetic biology to engineer simple and complex biological systems has the potential to disruptively innovate the development of vaccines, therapeutics, and diagnostics. Integration of molecular-level structure and dynamics, along with multiscale experiments with advanced computational techniques, enables a functional, systems-level description of pathogen-host interactions. For example, optical imaging, including fluorescence microscopy, provides dynamic information on microbial and cellular attachment and viability. However, mechanistic insights require tools, such as scanning probes and electron microscopes, that capture molecular structure and biological detail. Moreover, additional techniques can provide further complementary information, such as atomic force microscopy measurements in liquid environments, room temperature X-ray crystallography, and helium ion microscopy. Data from these diverse experiments must be assimilated and analyzed and require concomitant development of models and simulation across time and length scales to enable synergistic interactions between theory and experiment.

Scientific computing and experiments in biopreparedness and response rely heavily on data that frequently have access constraints due to national security, personal privacy, or industrial confidentiality concerns. Developing the fundamental theories and tools for rapidly utilizing access-constrained data will significantly increase predictive capabilities of computational models and the speed at which researchers can effectively respond. Of particular importance are advances that allow research teams to effectively harness near-term exascale computing systems as well as future generations of leading-edge computing environments—advances that were demonstrated through the DOE-led COVID-19 High Performance Computing Consortium (covid19-hpc-consortium.org).

6.4 Scientific Challenges

Scientific Challenge 1: Bridging the Fundamental Gap Between Fast and Efficient Data Science and Demanding Experimental Approaches

DOE's pandemic support delivered through NVBL was grounded in the core capabilities of fundamental science, which are based on the use of large-scale experimental facilities and leading-edge computing. In each area, DOE scientists carried out the largest computing campaigns possible today and combined them with the most demanding experiments. Pushing the boundaries of state-of-the-art in both computing and experimentation revealed that a fundamental gap exists between the two approaches. Bridging this gap would create faster, more efficient feedback loops, necessary for developing more effective epidemiology forecasts, personal protective equipment, pandemic diagnostics, and therapeutics. The future requires a framework that supports a productive feedback loop between novel computational and experimental modalities on a drastically shorter time scale, enabling rapid responses to emerging biothreats. In this framework, several key scientific challenges must be addressed, in particular:

- Experimental research and validation are slower than the rate at which computational methods can suggest new and promising research directions. A fundamental change in how experiments are prepared (e.g., sample synthesis) and performed (e.g., throughput) is required for them to match computational methods. Additionally, human-AI-facility collaboration is needed to steer optimized autonomous experimental workflows, thereby enabling experiments to increase contributions to the vast amounts of data and metadata required for AI-based data science.

- A significant gap exists between the speed of data collection and sample synthesis. For example, during the past 2 years, sample availability—not user facility beamtime—limited the rate at which viral protein structures and proteins complexed with other proteins, drugs, drug fragments, or antibodies could be solved. Investments in sample preparation and sample libraries are critical for addressing future pandemics. Researchers have taken two complementary experimental approaches to disrupting SARS-CoV-2 replication and tackling the COVID-19 pandemic. Some have tried to determine the structure of every SARS-CoV-2 viral protein and biochemically active complex with and without potential inhibitors and antibodies, while others have used fragment screening against a particular viral protein target, such as the SARS-CoV-2 NSP3 macrodomain (Schuller 2021). In the United Kingdom, the Diamond Light Source (DLS) coupled with the Pan-Dataset Density Analysis (PanDDA) method (Pearce et al. 2017) identified small molecules bound to proteins with high-throughput screening. One DLS beamline produced 79% as many SARS-CoV-2 related viral protein structures as all the macromolecular crystallography beamlines at the DOE light sources. Over 80% of the structures from DLS came from the beamline that supports high-throughput drug fragment screening (Krojer et al. 2017). DLS' success with this approach underscores a critical capability gap in U.S. user facilities.
- Data science hardware and software must also adapt to the ever-increasing data volumes created by experiments and observations. This adaptation would enable real-time data interpretation and greater data usage for time-critical information extraction and decision-making during the experimental process. These critical building blocks will facilitate autonomous discovery, aid decision-making, and, ultimately, make possible a joint human-AI-facility collaboration in research strategy development and execution.
- The output quality of computational models and methods relies on data and metadata quality. Experimental-computational feedback loops must be able to rapidly extract sufficient information from multiple data streams to intelligently guide a smaller or more focused set of experiments or model scenarios. However, exquisite, information-dense datasets (mostly from well-planned experiments) are limited in availability and quantity, while information-light (but not uninformative) data are more readily available in large quantities. New methods are necessary to extract key insights from information-light data sources to efficiently combine and integrate them with information-dense datasets. Another persistent challenge is the ability to integrate heterogeneous experimental, observational, and computational data across scales and modalities in an easy, verifiable way to rapidly extract key information. This core capability is essential for many biopreparedness-related projects, but especially for those focused on the design of new therapeutics or materials.
- Underpinning this framework is a need for robust cross-validation methods of heterogeneous experiments, data, and models to ensure validity and veracity of the scientific research conducted.

Scientific Challenge 2: Designing Qualitatively Different Pathogen Characterization Experiments to Strengthen Biopreparedness

Future biopreparedness will be propelled by new experimental and computational techniques, as well as a systems approach to integrating complex heterogeneous data. In fact, as new workflows seamlessly combine synthetic systems, advanced characterization, and studies of actual pathogen interactions, the classical distinction between experiment and theory will seem increasingly quaint and outdated. Advanced computational models will integrate systems biology with mechanistic modeling to represent and predict properties and behavior. Two new modalities have the potential to be highly impactful: (1) systems biology and synthetic biology (see sidebar p. 34), and (2) new integrative analytics linking molecular structure, chemical properties, and biological activity.

Systems Biology Supports Development of Synthetic Tools for Viral Research

Systems biology leverages techniques such as microbiological genomics, transcriptomics, proteomics, and metabolomics to investigate the complex interaction of a virus, cell, or organism in a holistic manner. Synthetic biology is a combination of physics, engineering, molecular biology, and cell biology to design and construct molecular components in simple or complex combinations for applications (*Nature* 2010; Khan et al. 2022; Tournier and Kononchik 2021). Systems and synthetic biology are intertwined. Systems biology provides the information needed for synthetic biology tool development, thereby enabling the manipulation of biological systems to better understand their complexity (Liu, Hoynes-O'Connor, and Zhang 2013). In the case of viruses, synthetic biology can be used to rapidly produce attenuated recombinant viruses to study specific properties without the associated risks of working with fully virulent viruses. Using both synthetic and systems biology, each gene of a virus and of target cells can be manipulated to elucidate which genes and gene mutations contribute to transmission and/or pathogenesis (Khan et al. 2022; Tournier and Kononchik 2021).

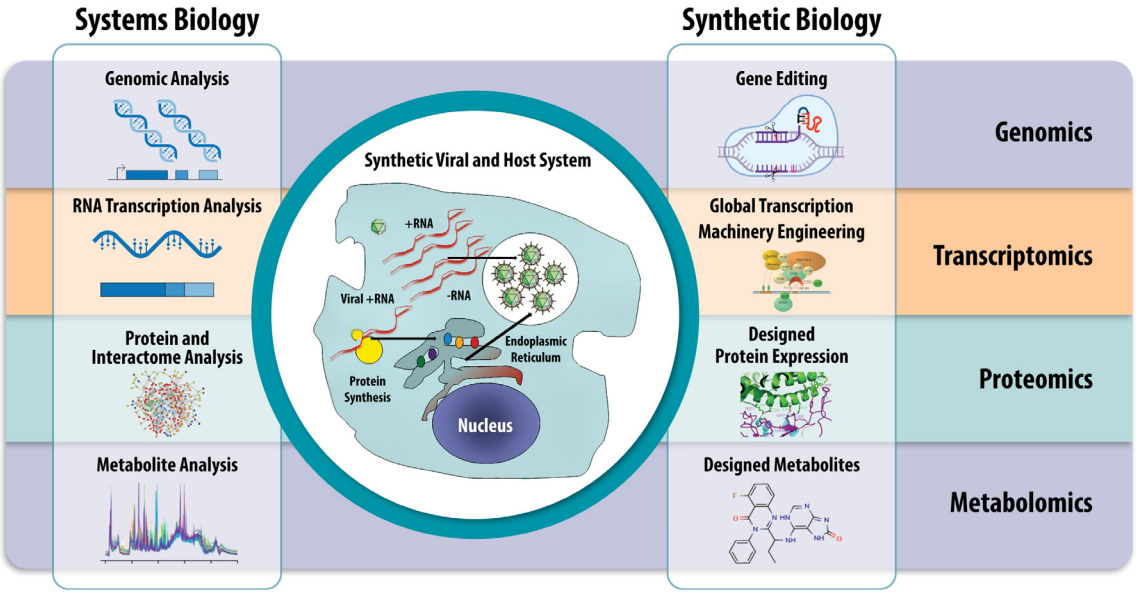

Fig. 6.3. Systems biology provides the information necessary for synthetic biological tool development supporting viral research. Synthetic biology tools can be used to significantly reduce the associated risks of working with pathogenic systems, producing an environment in which it is safe to explore the complexities of the virus.

[Image credits: Synthetic viral and host system courtesy Wikimedia Commons, protein and interactome analysis reprinted under Creative Commons License Attribution 4.0 International (CC BY 4.0) from Müller-Linow, M., et al. 2008. "Organization of Excitable Dynamics in Hierarchical Biological Networks," *PLoS Computational Biology* **4**(9), e1000190. DOI:10.1371/journal.pcbi.1000190. Metabolite analysis reprinted by permission from Springer Nature from Guijas, C., et al. 2018. "Metabolomics Activity Screening for Identifying Metabolites that Modulate Phenotype," *Nature Biotechnology* **36**, 316–20. DOI:10.1038/nbt.4101, ©2018. Gene editing reprinted under Creative Commons License Attribution 4.0 International (CC BY 4.0) from Khan, A., et al. 2022. "Combating Infectious Diseases with Synthetic Biology," *ACS Synthetic Biology* **11**(2), 528–37. DOI: 10.1021/acssynbio.1c00576. Global transcription reprinted by permission from Alper, H., et al. 2006. "Engineering Yeast Transcription Machinery for Improved Ethanol Tolerance and Production," *Science* **314**(5805), 1565–568. DOI: 0.1126/science.1131969. Designed protein expression reprinted by permission from Springer Nature from Shang, J., et al. 2020. "Structural Basis of Receptor Recognition by SARS-CoV-2," *Nature* **581**, 221–24. ©2020. Designed metabolites reprinted under Creative Commons License Attribution 4.0 International (CC BY 4.0) from Yu, Li et al. 2017. "Next-Generation Metabolomics in Lung Cancer Diagnosis, Treatment and Precision Medicine: Mini Review," *Oncotarget* **8**. DOI: 10.18632/oncotarget.22404.

Plug-and-play platform technologies are essential for quickly adapting to any pathogen of interest and ensuring a robust response against any future biothreat. As such, research and development (R&D) is necessary to establish a synthetic systems biology toolkit that is not only adaptable to any potential pathogen but can also be used modularly to characterize host-pathogen interactions resulting in virulence and spread through a population. The resulting detailed pathogen characterization will inform development of modeling and surveillance tools, assays, and sensors for rapid detection and diagnostics. It will also facilitate numerous countermeasures needed to combat evolving pathogens capable of causing a worldwide pandemic.

In addition to pathogen characterization, synthetic systems biology platforms can be used to (1) engineer biological organisms and biological products, (2) develop tools for detection and diagnostics, (3) design materials needed to reduce transmission, and (4) devise novel countermeasures with increased safety and efficacy. Each aspect is finely tuned so that only virulent pathogens are detected, therapeutic agents are delivered specifically to the target organ, and dosage is controlled (Khan et al. 2022; Vickers and Freemont 2022).

For disease characterization and drug testing, *in vitro* and *in vivo* tests are required. To this end, synthetic systems biology can be used to engineer microfluidic devices that mimic the targeted organ's microenvironment and generate relevant animal models with human properties responsible for disease. The field of synthetic systems biology and its associated tools enable almost limitless possibilities in terms of engineered organisms for production of biodegradable polymers, development of patient-specific therapies, and manufacture of medicine and other tools necessary for managing emerging pathogenic diseases.

Furthermore, comprehensive pathogen description necessitates integrative analytics that link molecular structure, chemical properties, and biological activity. A complete understanding of pathogen-host interactions requires determining mechanisms across length scales—from molecular fragments to the size of host cells—and time scales—from molecular vibrations to pathogen life cycles. Optimal imaging, X-ray and neutron diffraction, light scattering, imaging, and cryo-electron tomography are powerful tools, but each comes with physical limitations of sensitivity, field of view, and sample preparation requirements. Detailed pathogen characterization depends on correlating molecular locations and conformations across these different modalities. However, no single biomarkers—endogenous or exogenous—are currently readily available for such multimodal correlations. Therefore, new algorithms are needed to integrate heterogeneous data that will produce a complete pathogen description.

Scientific Challenge 3: Building Models with Constrained Data

Computational science in support of biopreparedness and response requires integrating a broad range of heterogeneous data to enable more effective predictive models for epidemiology, diagnostics, and therapeutics. Much of this data is distributed, and access is often severely restricted due to the data's proprietary nature, national security restrictions, personal privacy, or industrial confidentiality concerns. New experimental instruments, sensor technologies, and large-scale observational efforts have produced an inundation of data-generation technologies. This barrage has fueled explosive growth in the sheer volume and complexity of data generated across the DOE complex and beyond, raising concerns about the efficacy of existing data management tools and data movement between stakeholders and across computing resources.

Data with sensitive or proprietary access constraints come with legal, administrative, and policy processes that often require months to years to resolve. This timeline significantly limits the ability to predict and respond in a timely and efficient way to emerging biological threats. Research aimed at creating a comprehensive framework for securely analyzing global-scale data is necessary to support timely action during an emerging crisis. Federated and privacy-preserving data analytics, AI, and machine learning (ML) offer unique capabilities to support collaboration among the scientific community while addressing data access constraints. Federated learning allows multiple data stakeholders to collaborate in training large-scale, robust computational models without sharing data, while privacy-preserving AI/ML places further emphasis on

protecting stakeholder data. In the high-performance computing (HPC) domain, federated learning offers unique opportunities for better coordination across experimental facilities and federal agencies while leveraging the computing resources available across the DOE complex.

In this context, the following key scientific challenges have been identified:

- Developing theoretical foundations for extreme-scale encryption technologies that are highly scalable (i.e., size of data, speed of access and analysis) and easily integrated into computational models, workflows, AI, uncertainty quantification (UQ), and data transfer methods.
- Creating mathematical foundations and computational technologies that mitigate data access and sharing constraints, using data *in situ*, and building surrogate data models via knowledge distillation to alleviate both security and large data movement issues.
- Building distributed data ecosystems integrated with leadership HPC capabilities to provide more effective and efficient access to shared data without movement of that data.
- Designing next-generation data curation, data sharing, and data preservation technologies to support collaboration and documentation of data provenance.
- Investing in R&D for novel UQ that can integrate and analyze heterogeneous and multimodal data of variable quality to derive robust, trustworthy computational and AI models for prediction and decision support.

Capabilities Required to Address These Challenges

DOE facilities specialize in different aspects of molecular mechanisms, systems biology, surveillance, and testing. Examples include DOE's Joint Genome Institute for genomics; the Environmental Molecular Sciences Laboratory for transcriptomics, proteomics, and metabolomics; and DOE Office of Science light and neutron facilities for structural and imaging analyses. However, many of these facilities lack infrastructure for biosafety level (BSL) 2 and 3 containment. The absence of this capability impairs the ability to study host-pathogen and pathogen-material interactions using sophisticated techniques, such as cryo-electron microscopy (cryo-EM), small-angle scattering (SAS), reflectometry, and tomography. Examining these interactions requires the ability to move experimental and model systems between containment levels while preserving sample stability and integrity. Current methods for inactivating samples can alter their physical, chemical, and biological properties in ways we do not fully understand. Scientific or procedural methods for moving samples between containment levels or other biosafety risk management approaches would enable full utilization of DOE user facilities to mitigate a future biological event. Presently, only a few X-ray beamlines in the world are rated for BSL-3 level containment, including one at the Advanced Photon Source in the United States and one at the Diamond Light Source in the United Kingdom. Furthermore, the United States has no high-resolution BSL-3 cryo-EM facilities. As advancements in imaging methods proceed, a high priority will be placed on ensuring that biocontainment facilities can support sample analysis.

Along with the need for SAS and reflectometry (both neutrons and X-rays) to study conformational changes and pathogen-membrane interactions, new methods are also required to appropriately contain or inactivate samples of pathogenic origin while maintaining sample integrity. Moreover, strong interfacility interactions and collaborations will help accelerate R&D for safe sample inactivation and/or transfer across various imaging modalities and facilities.

In addition to containment demands and sample-sharing abilities across platforms, we must overcome the huge time cycle disparity that corresponds to dissimilar experimental methods. This gap makes it difficult to seamlessly integrate all analytical modalities, to use one technique's feedback to guide experiments on a different approach, and to completely integrate experiments with computation.

Addressing complex biopreparedness workflows at scale necessitates a more rapid integration of experimental and computational life cycles. Such

an advancement requires better coordination, co-planning, and collaboration among DOE facilities and resources. Seamlessly integrating workflows depends on capabilities (through practices, software, and middleware) that can better manage the full scientific data life cycle—from acquisition, metadata, provenance tracking, dataset integration, and results to data curation and archiving. Effective management of this complete data life cycle includes developing policies, practices, and privacy-preserving technologies for sharing sensitive data across multiple facilities. By addressing user needs for rapid and scalable analysis through workflow automation and AI/ML, a full life cycle can be supported to provide federated access to all appropriate resources and data.

Addressing a future pandemic will likely require numerous researchers across the country to simultaneously work on related questions and use qualified, traceable materials so results can be compared and validated. Protein expression, purification, and crystallization, as well as design and production of biomimetic membranes and other model structures, are currently performed in parallel in multiple institutions with little coordination. Significant efficiency gains could be achieved by creating a framework that unites teams with the common goal of providing materials for a broad research community.

DOE computing facilities will play a central role in advancing the bioscience foundations needed for accelerated pandemic response. Current systems, architectures, and use policies are designed to support large-scale simulation campaigns. Supporting the integrated experimental-computational workflows proposed in this chapter requires new capabilities that can bring these leadership-class computing systems together into distributed data ecosystems. DOE computing facilities, connected to broadly distributed data and experimental facilities, would provide a unique national test bed for accelerating bioscience.

Identified as common themes throughout this report's earlier chapters, the crosscutting needs outlined above support critical advancements for addressing the next biological crisis. These themes intersect at accelerating the experimental-compute life cycle and point to additional research needs for automating this process. Key requirements for addressing future grand challenges will be (1) a systems approach that combines complex heterogeneous data taken at high-throughput facilities with autonomous experiments and (2) real-time modeling and simulation that can transform data into knowledge. DOE national laboratories, academia, and national user facilities have the foundational capabilities necessary to accomplish this research and, ultimately, accelerate future breakthroughs in bioscience and biopreparedness.

Chapter 7

Conclusions

Government investments in basic science continue to play a critical role in the life sciences revolution and the associated biological, measurement, and computational technologies contributing to this transformation. The impact of these investments was broadly evident in the DOE COVID-19 pandemic response, coordinated through the Office of Science National Virtual Biotechnology Laboratory (NVBL). In addition to demonstrating the impact of foundational DOE investments, NVBL made clear the importance of pivoting research capabilities to accelerate response support for a nationally significant event.

The U.S. COVID-19 response underscored the significant value of addressing scientific gaps well in advance of a biological event. The roundtable on "Foundational Science for Pandemic Preparedness" convened multiple agencies and representation from all DOE national laboratories to identify gaps and assess the current state-of-the-art. The roundtable also identified five previously discussed priority research opportunities to address biopreparedness gaps, build on existing DOE capabilities in basic science, and create new capabilities to meet both biopreparedness needs and DOE missions.

Addressing these scientific gaps will enable a biopreparedness and response revolution in support of a future with a globally connected detection network—a digital immune system—that (1) continuously monitors populations and the environment for anomalies of concern; (2) informs planning and response decisions with accurate models of disease spread, impact, and prediction; (3) initiates platform vaccines and therapeutics that can be rapidly customized to specific threats; (4) provides design options for simple-to-use, threat-agnostic personal protective equipment and decontaminants that neutralize, detect, and characterize biothreats; and (5) responds rapidly to address novel pathogens with an integrated scientific infrastructure. Furthermore, the underlying science and technology advances gained through these research opportunities will provide capabilities to not only enhance biodefense research areas essential for addressing and mitigating future biological threats, but also advance preparedness to tackle other crises impacting health, the economy, and security.

Appendix A

Roundtable Charter

Office of Science Roundtable on Biopreparedness and Response

Chartered by: The Office of the Deputy Director for Science Programs, in collaboration with Office of Science Programs, including the Offices of Advanced Scientific Computing Research (ASCR), Basic Energy Sciences (BES), and Biological and Environmental Research (BER).

Mode: A virtual plenary kickoff, followed by a two-week period of asynchronous virtual panel discussions and writing (supported by virtual collaboration tools) and a virtual closing plenary session, including presentations of report-outs by panel chairs and roundtable findings by co-chairs.

When: March 8, 2022, 12:00 p.m. – 4:00 p.m. ET: Plenary kickoff

March 15, 2022, 12:00 p.m. – 4:00 p.m. ET: Roundtable check-in and discussion

March 22, 2022, 12:00 p.m. – 4:00 p.m. ET: Wrap-up plenary

Planning Team: Chair and co-chairs, with Michelle Buchanan, Joseph Graber (BER), Thomas Russell (BES), Margaret Lentz (ASCR), Natalia Melcer, and Katie Runkles representing the Office of Science.

Program Committee: Chair and co-chairs, along with panel leads and co-leads will drive program planning and execution and will be responsible for producing the roundtable report.

Attendees: By invitation only, including representatives from national laboratories, universities, other federal agencies and departments, and industry.

Deliverable: A report identifying priority research opportunities, including specialized capabilities to support biothreat studies at user facilities, for the Office of Science by May 2022.

Motivation: DOE's response to the COVID-19 pandemic brought together expertise and capabilities across all 17 DOE national laboratories, including core capabilities in biological, chemical, physical, and computational sciences, and engineering, to form the National Virtual Biotechnology Laboratory (NVBL). The NVBL focused on five research areas including COVID-19 Testing, Molecular Design for Medical Therapeutics, Materials and Manufacturing, Epidemiological Modeling, and Viral Fate and Transport.[1] In addition, the capabilities of DOE's user facilities[2], including light and neutron sources, leadership computing facilities, nanoscale science research centers, and biology user facilities, were employed by the broader scientific community, including researchers from universities, industry, and other federal agencies.

The impact of DOE's capabilities in the fight against COVID-19 has been enormous. Vaccine developers relied upon DOE light sources to support the development of all three FDA-approved vaccines currently in use in the U.S. In addition to developing new sampling and analysis technologies, NVBL supported the FDA and CDC by validating the effectiveness of commercial COVID-19 tests. NVBL's Epidemiological Modeling team supported decision-makers at the local, state, and national levels to understand disease spread and the impact of closing restaurants, opening schools, and other of administrative decisions. The Viral Fate and Transport team also evaluated the spread of the virus in indoor and outdoor environments. The Materials and Manufacturing team, working with industry, rapidly

[1] science.osti.gov/nvbl

[2] www.energy.gov/science/science-innovation/office-science-user-facilities

developed new materials and manufacturing processes that addressed shortages in face masks, sample kit components, and ventilators, and generated over 1,000 new jobs. Finally, the Molecular Design team used both computational modeling and structural biology tools available at DOE's user facilities to identify promising candidates for therapeutic interventions.

This roundtable is being convened to gather information about the unique roles the Office of Science could play in addressing future pandemics and related crises, including identifying priority research opportunities and specialized capabilities needed to support biothreat studies at user facilities.

Roundtable Discussion Topics

- **Panel 1:** Surveillance, Testing, and Diagnostics
- **Panel 2:** Molecular Mechanisms, Systems Biology, and Therapeutic Development
- **Panel 3:** Epidemiological and Event Modeling for Response and Recovery
- **Panel 4:** Materials and Manufacturing
- **Panel 5:** Crosscutting Team: Facilities and Data

Appendix B

Roundtable Agenda

All times are Eastern. Sessions were held via Zoom.

Tuesday, March 8, 2022		
12:00 p.m. – 12:05 p.m.	Welcome	Harriet Kung, U.S. Department of Energy
12:05 p.m. – 12:10 p.m.	Roundtable Introduction	John Hill, Brookhaven National Laboratory
12:10 p.m. – 12:30 p.m.	Plenary 1: U.S. Department of Defense Perspective	Ron Hann, U.S. Department of Defense
12:30 p.m. – 12:50 p.m.	Plenary 2: National Institutes of Health Perspective	Susan Gregurick, National Institutes of Health
12:50 p.m. – 12:55 p.m.	Break	
12:55 p.m. – 1:15 p.m.	Plenary 3: Centers for Disease Control and Prevention Perspective	Joanne Andreadis, Centers for Disease Control and Prevention
1:15 p.m. – 1:35 p.m.	Plenary 4: National Virtual Biotechnology Laboratory Recent U.S. Department of Energy Activities	Stephen Streiffer, Argonne National Laboratory
1:35 p.m. – 1:40 p.m.	Break	
1:40 p.m. – 2:40 p.m.	Panel Discussion	Plenary Speakers
2:40 p.m. – 2:45 p.m.	Break	
2:45 p.m. – 3:45 p.m.	Panel Breakouts	Panel Leads
3:45 p.m. – 4:30 p.m.	Reconvene for Discussion	Co-Chairs and Panel Leads
4:30 p.m.	Adjourn	

Tuesday, March 15, 2022		
12:00 p.m. – 12:05 p.m.	Welcome and Opening Remarks	Co-Chairs
12:05 p.m. – 12:25 p.m.	Panel 1 Update: Surveillance, Testing, and Diagnostics	Panel Leads: Kristin Omberg, Pacific Northwest National Laboratory, and Monica Borucki, Lawrence Livermore National Laboratory
12:25 p.m. – 12:45 p.m.	Panel 2 Update: Molecular Mechanisms, Systems Biology, and Therapeutic Development	Panel Leads: Ben Brown, Lawrence Berkeley National Laboratory, and Marti Head, Amgen
12:45 p.m. – 1:05 p.m.	Panel 3 Update: Epidemiological and Event Modeling for Response and Recovery	Panel Leads: Sara Del Valle, Los Alamos National Laboratory, and Budhu Bhaduri, Oak Ridge National Laboratory
1:05 p.m. – 1:20 p.m.	Break	

1:20 p.m. – 1:40 p.m.	Panel 4 Update: Materials and Manufacturing	Panel Leads: Ilke Arslan, Argonne National Laboratory, and Brett Helms, Lawrence Berkeley National Laboratory
1:40 p.m. – 2:00 p.m.	Panel 5 Update: Crosscutting Themes	Panel Leads: Soichi Wakatsuki, SLAC National Accelerator Laboratory, and Jim Brase, Lawrence Livermore National Laboratory
2:00 p.m. – 2:45 p.m.	Discussion	Co-Chairs
2:45 p.m. – 3:00 p.m.	Break	
3:00 p.m. – 4:00 p.m.	Panel Breakouts	Panel Leads
4:00 p.m. – 4:30 p.m.	Reconvene for Discussion	Co-Chairs
4:30 p.m.	Adjourn	

Tuesday, March 22, 2022		
12:00 p.m. – 12:15 p.m.	Welcome and Opening Remarks	Co-Chairs
12:15 p.m. – 12:45 p.m.	Panel 1 Report: Surveillance, Testing, and Diagnostics	Panel Leads
12:45 p.m. – 1:15 p.m.	Panel 2 Report: Molecular Mechanisms, Systems Biology, and Therapeutic Development	Panel Leads
1:15 p.m. – 1:45 p.m.	Panel 3 Report: Epidemiological and Event Modeling for Response and Recovery	Panel Leads
1:45 p.m. – 2:00 p.m.	Break	
2:00 p.m. – 3:00 p.m.	Panel 4 Report: Materials and Manufacturing	Panel Leads
3:00 p.m. – 3:30 p.m.	Panel 5 Report: Crosscutting Themes	Panel Leads
3:30 p.m. – 3:45 p.m.	Break	
3:45 p.m. – 4:30 p.m.	Discussion	Co-chairs
4:30 p.m.	Adjourn	

Appendix C

Roundtable Participants

Chair

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Panel 2: Molecular Mechanisms, Systems Biology, and Therapeutic Development

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Panel 3: Epidemiological and Event Modeling for Response and Recovery

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Panel 4: Materials and Manufacturing

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Appendix D

Technology Status Document—Foundational Science for Pandemic Preparedness

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D1. Introduction

The natural or anthropogenic emergence of pathogens capable of causing diseases of epidemic or pandemic potential has been an ongoing threat to human health security throughout history (Madhav et al. 2017). During the Roman Empire, the plague, caused by the bacterium *Yersinia pestis*, killed an estimated 100 million people between 541 and 543. The influenza pandemic, dubbed the "Spanish Flu," resulted in approximately 500 million infections and 50 million deaths worldwide between 1918 and 1920. During the past century, increased human land use, globalization, migration, and climate change effects have contributed to the emergence of more infectious diseases around the world (Baker et al. 2021). In addition, growing resistance to antimicrobial drugs and antibiotics has also spurred the resurgence of several infectious diseases. The world has experienced outbreaks of cholera, plague, influenza, SARS, MERS, Ebola, Zika and other infectious diseases. The COVID-19 pandemic—with a current tally of 424 million cases and 5.8 million deaths worldwide (WHO 2022)—will not be the last outbreak to impact our way of life. Thus, concerted and well-integrated efforts are needed to strengthen our capabilities to detect, prevent, prepare for, respond to, and recover from biological incidents.

In 2018, the U.S. National Biodefense Strategy established an overarching plan and processes to coordinate biodefense efforts across the U.S. government to address natural, accidental, and deliberate biological threats affecting humans, animals, plants, and the environment (White House 2018). The current pandemic has reinforced the important roles of science and innovation for addressing such threats, including through the rapid development of mRNA vaccines made possible by many years of investment in the foundational science needed to create these new vaccines (Dolgin 2021). However, the COVID-19 pandemic has also illustrated that in addition to further coordination among federal, state, local, tribal, territorial, and private sector stakeholders, greater focus is needed on the science and technology required to effectively address future pandemics and biological threats. These needs are outlined in the Biden administration's recently released pandemic preparedness plan, "American Pandemic Preparedness: Transforming Our Capabilities" (White House 2021).

The U.S. Department of Energy's (DOE) national laboratories play important roles in supporting the U.S. biodefense enterprise through science and innovation. In response to the COVID-19 pandemic, DOE established the National Virtual Biotechnology Laboratory (NVBL) in March 2020. NVBL harnessed capabilities across all 17 DOE national laboratories for rapidly addressing needs in five research and development (R&D) areas: (1) materials and manufacturing of critical supplies, (2) molecular design for COVID-19 therapeutics, (3) COVID-19 testing, (4) epidemiological modeling, and (5) viral fate and transport (U.S. DOE 2021). NVBL made critical advances in these areas by leveraging DOE's world-leading experimental and computational user facilities and capabilities, such as light and neutron sources, nanoscale science and research centers, sequencing and biological characterization facilities, and high-performance computing facilities. DOE is also part of the COVID-19 High Performance Computing (HPC) Consortium that brought together the world's most powerful HPC resources to support COVID-19 research. Sustained efforts in biopreparedness research and innovation are needed to continue to respond to the current pandemic and address future biological threats.

The purpose of this technical status document is to help inform future pandemic preparedness research by providing a summary of the current state of the art as well as needs for strengthening biopreparedness in four areas: (1) surveillance, detection, and diagnostics; (2) molecular mechanisms, systems biology, and molecular therapeutics; (3) epidemiological and event modeling for response and recovery; and (4) materials and manufacturing. Developed by subject matter experts across DOE national laboratories, this report provides a general sense of the current state of the art and needs for each area, rather than a comprehensive analysis of capabilities and gaps. Although this document focuses on preparedness for pathogen-related biological events that impact human health, many of the foundational science capabilities developed for pandemic preparedness can also be applied to other emerging biological threats that could significantly affect humans, animals, plants, and the environment.

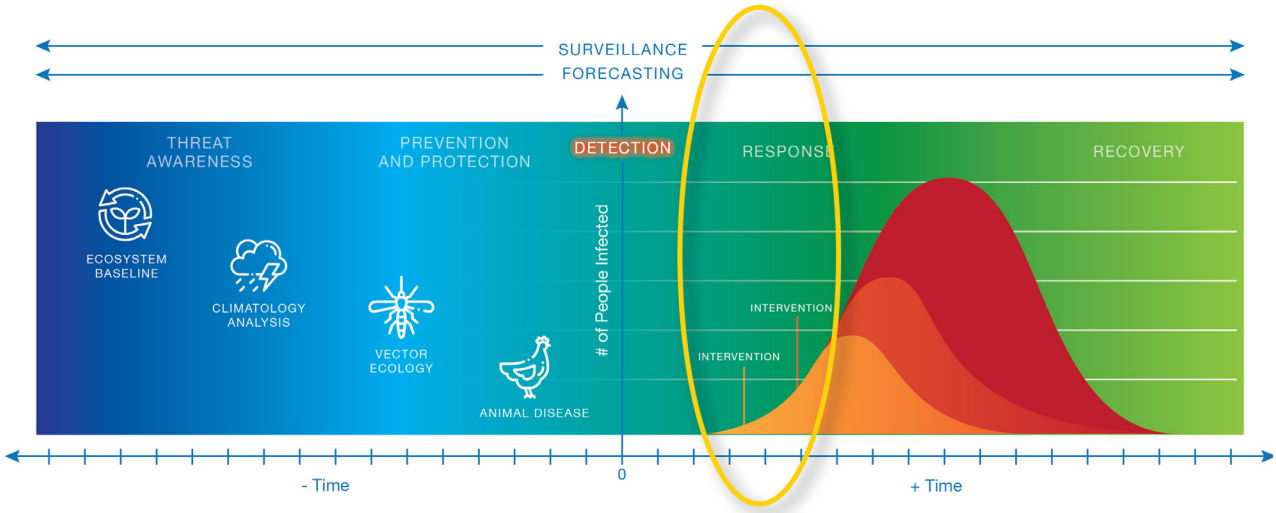

Fig. D1. Schematic representation of an outbreak timeline, irrespective of whether the event is natural, accidental, or intentional. Signatures in ecosystem processes, impacts of climate, changes in vector ecology, changes in zoonotic transmission, and other factors impacting the event occur before the event initiation (left of boom). Surveillance approaches (alongside forecasting and prediction) can be targeted to provide anticipatory information regarding such events. Detection technologies should be implemented before the event (before t=0) to prevent a biological event and have maximal impact on minimizing the peak of the outbreak curve. Other intervention strategies occur (right of boom) after evidence of infection and identification of countermeasure strategies. Surveillance tools and agnostic diagnostics can greatly help minimize the peak of any outbreak.

[Image credit: Los Alamos National Laboratory]

D2. Surveillance, Detection, and Diagnostics

Early recognition that a biological event is occurring (or will occur), and identification of the associated pathogens are cornerstones for preventing disease transmission and spread (Manore et al. 2019). Threat identification through surveillance and implementation of diagnostics and detection systems early in the biological event cycle are critical components needed to minimize downstream negative impacts (see Fig. D1, this page). Identification of both anticipated and unanticipated threats is also crucial for effective intervention against emerging pathogens, whether natural or anthropogenic. Thus, both pathogen-specific (targeted) and pathogen-agnostic (untargeted) approaches are needed to ensure the identification of all pathogens. In addition, a surveillance and identification system requires a centrally controlled, readily deployable, rapid, cost-effective architecture to be sustainable and effective at the community level (Stoto 2014; Bajema et al. 2021). While the requirements of an effective biodetection system for pandemic preparedness may seem daunting, seeking inspiration from natural systems that have effectively accomplished this undertaking may enhance our chances of success. Indeed, a natural architecture that satisfies all criteria described above is our own human immune system (Chaplin 2010; Delves and Roitt 2000).

The human immune system integrates (1) physical immunity (i.e., skin and hair) akin to the personal protective equipment (PPE, masks, gloves) used to prevent exposure to infectious agents; (2) innate immunity focused on early, rapid agnostic broad-based surveillance and identification of all pathogens, known and unknown; and (3) adaptive immunity designed for highly specific, targeted pathogen identification coupled with strategies for infection mitigation and long-term prevention. All three elements operate at different time scales and have distinctive purposes, and the effective communication and integration between the elements provides a time-tested and sustainable system aimed at protection from invading pathogens. Mimicking this layered strategy can help realize an effective biosurveillance, testing, and identification approach for pandemic preparedness. This layered strategy must also consider all pathogens—known and

unknown, bacterial and viral, anthropogenic and natural—so the basic backbone of scientific investments and response infrastructure can be equally applicable to present and future pandemics.

Thus, pandemic preparedness requires effective surveillance to provide early warning and monitor biological event progression. Surveillance strategies, which involve agnostic (preferably) and/or specific approaches for early infectious disease identification, can be applied to pathogen identification in either environmental samples or in human or animal hosts. These strategies also include measurements of additional signatures, indicators, and parameters related to an emerging biological event. The U.S. National Biosurveillance Strategy recognizes the importance of surveillance and calls out the need to develop "a well-integrated national biosurveillance enterprise that saves lives by providing essential information for better decision-making at all levels" (White House 2012). Surveillance technologies should be easily deployable, simple, and readily usable for community monitoring, cost-effective, and provide rapid answers. To address both previously anticipated and unanticipated threats, surveillance technologies should include measurements that are agnostic to the causative pathogen.

Surveillance should be followed by more specific and tailored pathogen identification to facilitate effective therapeutic intervention and event tracking. Thus, targeted diagnostics are intrinsically important for pandemic preparedness, and much of the U.S. government's investment has centered on pathogen-specific approaches. The World Health Organization's (WHO) Research and Development (R&D) Blueprint for Epidemic Preparedness highlights the need for rapid and early diagnostics for early identification of pandemic and epidemic threats (WHO 2016). The report also identifies pathogens associated with global risk and recommends focus areas for diagnostic development. However, no available diagnostics exist for some of these pathogens, and for others, the diagnostics are only sparingly available at regional and reference laboratories. Targeted diagnostics that can be readily used at home, in local clinics, and in facilities can greatly help with timely decision-making and curb further spread of infection.

The suite of diagnostics and detection assays available or in development for COVID-19 is extensive. However, even today (February 2022), many of these diagnostics are not readily accessible, cost-effective, rapid, sensitive, or accurate. As a result, our ability to facilitate on-the-spot decision-making is limited (Vandenberg et al. 2021). Overcoming these limitations will call for strategic investments not only in diagnostics and detection approaches, but also in data integration and capture platforms (that include sample processing, measurement, and analysis methods). Indeed, such approaches are not only valuable for pandemic preparedness. Global health efforts, warfighter support, biothreat and biowarfare detection also require systems that are rapid, deployable, pathogen-agnostic and specific (layered), cost-effective, and easy-to-use. Delivering on these requirements involves the agile integration of multidisciplinary scientific approaches, including, but not limited to, bioscience, microbiology, engineering, nanoscience, materials science, physics, chemistry, informatics, and high-performance computing. Meeting diagnostic and detection requirements also necessitates the ability to transition between fundamental and applied science and to incorporate field-testing and validation of the technologies. DOE laboratories have many capabilities in these areas that could be harnessed to strengthen surveillance, detection, and diagnostics.

D2.1 Current Capabilities and State of the Art: Surveillance, Detection, and Diagnostics

Biodetection poses challenges, especially in the context of pandemic preparedness and biodefense, because in addition to anticipated threats, these technologies ultimately aim to identify even unprecedented or previously unknown pathogens. Microbes are all around us, and the distinction between pathogens and nonpathogens can be extremely subtle (Childs et al. 2007). Differences in a few percent of the genome sequence, or variable expression of a virulence determinant representing a tiny fraction of the total cell protein content, can make the difference between a harmless environmental microbe and a virulent pathogen. Further, pathogen emergence cannot be assessed only from quantifiable increases in the detection of a microbe. For instance, sudden changes in the amounts

of a microbe in a given sample can indicate emergence of a pathogen or simply a transient response to changing environmental conditions. Indeed, chemical and physical properties of pathogens and nonpathogens are not always markedly different from each other, and pathogenic determination of a microbe depends on the host. For example, Ebola virus, carried routinely by bats, is a deadly pathogen to humans (Jacob et al. 2020). In addition, endemic pathogens in certain geographical areas of the world can cause deadly outbreaks in naïve populations, and with increasing global migration and climate change, the risk of such epidemics is higher. Increased global travel and economic exchange play a role in such events. The recent challenges associated with outbreaks of Chikungunya (Del Valle et al. 2018) and Zika (Kobres et al. 2019) viruses and the current COVID-19 pandemic illustrate how the global movement of people can rapidly result in international outbreaks.

At this juncture, it is important to define the terms *detection* and *diagnostics*. Detection refers to the identification of a pathogen or associated signatures in environmental samples, such as air, water, soil, fecal matter, food, and other sources. Diagnostics refers to the identification of pathogen infection or exposure in a human or animal host to understand health status and inform treatment. Thus, diagnosis comes with the added challenges of (1) biological sample choice, (2) identification of signatures indicative of active infection vs. exposure, and (3) the requisite sensitivity to identify signatures in the biological sample background. The technological requirements of biodetection and diagnostic strategies can be different based on their applications for clinical intervention or surveillance. Indeed, while both detection and diagnostics are critical for pandemic preparedness, a technology suitable for one application may not always be used with the other. Additionally, sample preparation requirements will depend on the sample type and concentration of pathogen (or related signature) in the sample. Furthermore, streamlined approaches are critically needed to assess accuracy of available diagnostics and detection methods. The development of a method to identify an unknown threat suffers from the lack of gold-standards and effective benchmarking methods. However, the development of systemic standards and evaluation pipelines can help circumvent this challenge.

A broad range of methods have been developed for detection and diagnosis of biological agents. Such methods can be categorized as targeted or untargeted.

Targeted Methods

Targeted methods rely on a specific molecular recognition event, typically a protein-binding event or nucleic acid hybridization, to trigger signal reporting. These methods typically use a targeted ligand that is pathogen-specific, such as an antibody, aptamer, nucleic acid probe, and others. Thus, targeted methods can achieve excellent sensitivity and specificity, but they require advanced knowledge of agent properties to be detected. Most targeted detection and diagnostic methods seek to identify either nucleic acid or protein signatures of interest in a given sample, as described in the following sections.

Nucleic Acid-Based Methods

The quantitative polymerase chain reaction (qPCR) method used for SARS-CoV-2 testing is the gold standard of targeted methods, and qPCR is based on the identification of pathogen-specific DNA or RNA signatures. qPCR represents perhaps the ultimate in sensitivity; theoretically, the method can detect a single copy of a pathogen genome if present in a sample. However, the standard approach to qPCR is expensive, laboratory- and skill-intensive, and operationally complex. qPCR is also exquisitely specific, and it can be tuned to detect an agent only when an exact match with a pathogen DNA/RNA signature is found. Thus, methods other than PCR are needed to make the initial discovery of a new and emerging pathogen. Indeed, the current pandemic clearly demonstrates that any diagnostic approach should keep pace with the evolving nature of the causative pathogen. Some assay degeneracy can be built into PCR through assay design analytics to allow amplification and detection of DNA sequence ranges, but this feature requires consistent redesign and upkeep. Furthermore, the success of qPCR methods depends on sample quality and the presence of sufficient target material and inhibitory compounds (Kralik and Ricchi 2017; Kevadiya et al. 2021).

Alternative targeted methods to qPCR have been developed for detecting pathogen DNA and RNA in a sample of interest. Some of these methods can provide advantages, such as reduced burden of sample preparation, simplified instrumentation, and/or more rapid detection. These methods include isothermal DNA/RNA amplifications, such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA). Moreover, CRISPR-based detection methods have also been developed using a variety of effector proteins and specific RNAs. Although, in general, these methods are not nearly as well-developed as qPCR, and further fundamental research is needed for them to reach their full potential as qPCR alternatives for highly sensitive and specific detection.

Protein-Based Methods

The other general category of targeted methods relies on molecular recognition of proteins, typically using antibodies or other highly specific affinity reagents that bind to a distinctive protein marker. Immunoassays are generally orders of magnitude less sensitive than DNA/RNA amplification methods such as qPCR, but great progress has been made in deploying immunoassays in the form of simple, easy-to-use formats, such as lateral flow assays (e.g., at-home rapid antigen tests for COVID-19). Thus, immunoassays are much more conducive to personalized medicine applications, which is an important consideration for technologies that can be used during a pandemic (Koczula and Gallotta 2016; Aydin 2015; Galipeau et al. 2020).

A major challenge for immunoassays is the need to develop affinity reagents (typically antibodies) for each new target of interest. Traditionally, this process involves isolating a distinctive antigen for the pathogen, then injecting this antigen into laboratory animals and relying on the animal's immune system to generate candidate antibodies, which can then be further purified, isolated, cloned, and expressed. Newer methods for developing affinity reagents utilize *in vitro* selection and rapid library-based selection approaches, such as phage display and yeast display (Alfaleh et al. 2020; Könning and Kolmar 2018; Gray et al. 2020). A diversity of antibody formats, including single-chain variable fragments, single-domain antibodies, and nonantibody formats such as aptamers (nucleic acid-based), are enabled by *in vitro* selection methods. Moreover, progress has been made in the *de novo* design of stable peptides and affimers (small proteins) as affinity reagents (Bhardwaj et al. 2016). Small molecule ligand libraries have also been computationally designed and could be screened computationally for binding affinity to a target of known structure. In any case, the process is time-consuming and requires a high degree of skill. Many ligands selected using these processes fail to perform with equal efficiency in physiologically relevant matrices such as clinical samples, which is a major challenge. Reducing the timeline for affinity reagent development, especially without the use of animals, is an important need for improving immunoassay development. Furthermore, the design and development of stable and reversible affinity reagents would enable long-term use in surveillance approaches, including but not limited to wearables. Methods to improve the sensitivity of simple immunoassay formats for diagnostics, such as lateral flow assays, to be on par with molecular methods would dramatically improve their utility in pathogen screening.

Multiplexing Known Targets

The simultaneous use of multiple anticipated signatures increases the reliability and capture efficiency of targeted methods in the event of unprecedented and unanticipated events. Highly multiplexed detection platforms can also be used to simultaneously screen for a wide range of pathogens. Thus, the ability to multiplex is a critical component of any detection strategy being considered for pandemic preparedness. PCR-based techniques can multiplex multiple targets through a combination of targeted primers and relevant probes. Nucleic acid microarrays have been developed to screen for many thousands of sequences but do not have the sensitivity of PCR and require DNA amplification prior to hybridization to the microarray. Advances in multiplex protein measurements over the past decade have also been tremendous. For example, flow cytometric methods, planar microarrays, quantum dots for multiplex fluorescence assays, nitrocellulose functionalized microfluidics, paper-based assays, and multiplex immunoassay platforms have been developed for detecting multiple known targets (Basha et al. 2017; Mukundan et al. 2010).

Untargeted Methods

Untargeted methods, by contrast, encompass a broad array of methods that interrogate various chemical and physical properties of bioagents. While these methods may be biased toward certain categories of agents, they are open-ended in that a single test or measurement may detect and identify many different agents. Some methods are suitable for broad-based identification of biological agents and pathogens, but they are associated with limited specificity. Examples include certain optical methods based on light scattering and fluorescence spectroscopy (Huffman et al. 2020). Such methods can detect aerosolized biological agents and identify that something of biological origin is present in a plume, but with minimal information regarding the type of biological material present. Optical aerosol detection methods can also be rapid (i.e., real-time detection, within seconds) and serve a role in early warning and surveillance systems. For example, they can be used to indicate the potential presence of an aerosolized biological threat. However, the sensitivity of these methods can be impacted by other components in the sample. Further, the specificity of these methods is traditionally poor, and they have minimal ability on their own to distinguish between dangerous bioagents and innocuous environmental organisms. That said, recent advances in high-performance computing and the use of artificial intelligence and machine learning (AI/ML) algorithms to decode complex data sets, such as spectroscopic data sets, have begun to unravel specific signatures that are difficult to interpret manually. The use of these technologies, in concert with broad-based spectroscopic and optical sensors, can greatly enhance the usable information gained from such platforms. In addition, it may be possible to combine multiple orthogonal data streams or pieces of data to improve detection of a suspected biological event.

Beyond this, untargeted methods can be broadly categorized as (1) high-content spectroscopic and spectrometric methods and (2) sequencing methods.

Spectroscopic and Spectrometric Methods

Spectroscopic measurements use electromagnetic radiation to interrogate samples, whereas spectrometric techniques analyze molecules by their fragmentation patterns, which are typically measured by mass spectrometry. More broadly, spectroscopy has also been described as the measurement of the absorption or emission of light and other radiation for obtaining information about a system or its components (IUPAC 1997). Some of the methods in these categories are vibrational spectroscopy (e.g., Raman spectroscopy [Serebrennikova et al. 2021]), laser induced breakdown spectroscopy (LIBS), surface plasmon resonance spectroscopy (SPR), and mass spectrometry (Duriez et al. 2016). Individual measurements may be made quickly in seconds or minutes, although some cases may require extensive sample preparation or purification prior to the measurement.

Specimens may comprise complex environmental samples, aerosols, or organisms that have been isolated by culture or other methods. In the case of mass spectrometry, specimens can include elements of the surrounding environment for analysis, such as using the headspace above a sample for volatile compound analysis. In either case, subjecting a biological specimen to a spectral analysis generates a complex spectrum or set of peaks. In most cases, it is not possible to assign individual peaks in a spectrum from a biological agent to individual chemical species. Nor is it currently possible to predict, from first principles, what the spectrum from a particular biological agent would look like. Rather, the analytical approach is akin to matching spectral fingerprints to a database. Feature extraction and ML are critical to this approach. A challenge for spectroscopic methods is that the ability to detect an agent is only as good as the library or database used for training the algorithm.

Areas for further research include rapid sample preparation methods, more comprehensive reference libraries, enhanced spectral resolution methods, and improved algorithms for spectral feature identification, potentially including interpretable models or first-principal modeling. As noted earlier, advances in computational methods including AI/ML technologies can help expand the capabilities of spectroscopic characterization for challenging applications, such as agnostic biodetection. Indeed, in space programs (e.g., the ChemCam and SuperCam sensors on Curiosity and Perseverance rovers on Mars), spectroscopy has been the go-to signature identification technique in an

environment lacking advanced information (Wiens et al. 2021; Cousin et al. 2022; Wiens et al. 2012). Similarly, many national security applications and remote sensing strategies use spectroscopic methods. In this context, spectroscopic and spectrometric methods are routinely used for complex sample interrogation. Further investments in data analytics, engineered solutions, and materials and fabrication technologies, in concert with bioscience innovation, can help advance these capabilities toward achieving agnostic biodetection.

Sequencing Methods

Sequencing currently represents the most definitive and developed untargeted method for biodetection, especially for identification of multiple pathogens, although more work is required to develop simpler and lower cost sequencing and analysis technologies to enable broad-based application to surveillance and early diagnostics applications (Byron et al. 2016). Indeed, the ongoing COVID-19 pandemic saw the broad application of sequencing methods to clinical diagnostics and surveillance, an especially critical application with the advent of multiple variants of the pathogen (John et al. 2021; NLM 2022). To date (as of February 2022), more than 2.5 million SARS-CoV-2 sequences have been uploaded to GISAID, a public database for influenza virus sequence data, with weekly additions of at least 200,000 sequences.

A variety of sequencing technologies exist (Slatko et al. 2018): traditional low throughput but highly accurate Sanger sequencing, massively parallel "next generation" additive sequencing methods (e.g., Illumina sequencing), very rapid but low accuracy Oxford Nanopore sequencing, and others. Each suite of technologies differs in chemistry, sequencing methods for library preparation, and instrumentation. Regardless of the mechanism, sequencing methods produce a readout of DNA or RNA sequences present in a sample, with no advanced knowledge of the organism required. Thus, sequencing is the one method capable of both detection and *de novo* identification of novel bioagents. However, significant research is needed to understand the relationship between sequence and function and to understand pathogenicity and level of concern associated with an uncharacterized organism sequence. In addition, sequencing from anything other than a pure sample still requires extensive sample preparation and sophisticated bioinformatics analysis to categorize and identify sequence data. Most methods typically analyze either DNA or RNA, but not both simultaneously, due to compositional bias. Most sequencing is conducted on short fragments, resulting in challenges with accurate computational assembly of the fragments to generate a complete DNA or RNA sequence. Longer-read strain-resolved sequencing is required to accurately identify new variants or pathogens. Bioinformatic tools for sequence characterization and identification have also evolved significantly and use of the associated algorithms is simpler than ever before (Li et al. 2017; Cohn et al. 2018).

Sequencing from complex or environmental samples is still very challenging due to the diversity of biosignatures that may be present (Byron et al. 2016). Large data sets with embedded sequences from many sources are difficult to characterize and classify. Bioinformatic algorithms must be tuned to balance sensitivity and accuracy, and the high overlap between genomes of pathogens and near-neighbors can create false-positive detections when tuned for very high sensitivity. Detecting pathogens in human diagnostic specimens such as blood is also complicated by the overwhelming presence of human genomic material. Despite the potential as an agnostic diagnostic, inferring and identifying presence of a novel pathogen based on a never-before encountered sequence is still in its infancy. Thus, sequencing today is largely used for characterization and is often preceded by epidemiological cues, as well as the long and laborious process of culture and isolation of a pathogen from clinical specimens. More research is required to simplify sequencing and associated bioinformatic algorithms and understand the relationship between sequence and function to make sequencing more suitable for pandiagnostic applications.

Sample Processing

Sample processing is one of the major limitations to effectively deploying detection and diagnostic strategies. Depending on the sample under consideration (e.g., blood, urine, food, water, soil, sewage, etc.) and the nature of the signature being interrogated, sample processing can be complex and time consuming, often complicating results. Further, processing a sample to evaluate one type of signature often removes all others,

greatly diminishing information yield. Processing samples for the release of nucleic acid or protein signatures has been relatively streamlined, but pandemic preparedness requires more field-ready methods that can separate and capture biochemically disparate signatures. Sample preparation must retain the integrity of the target analyte, whether it be a protein or nucleic acid. Some teams have started designing and evaluating microfluidics and laboratory-based sample processing solutions, some of which are already commercially available (Sonker et al. 2017; Lenz et al. 2021). But this area requires additional work and integration of existing sample processing methods with detection technologies (Hernandes et al. 2017; Nichols and Geddes 2021).

Emerging Technologies

Beyond the current paradigms of targeted and untargeted methods, potential room exists for novel methods with additional features of interest, including:

Human Immune Recognition

This chapter's introduction discussed how the human immune system may serve as an inspiration for biodetection by cuing in on properties that are unique to pathogens. For example, specific functions or pathways are distinctive among classes of pathogens but are not present in innocuous microbes. Indeed, innate immunity has evolved to identify evolutionarily conserved signatures on all pathogens, making it the most robust agnostic diagnostic pipeline in existence. Methods based on immune recognition can target either the pathogen signatures recognized by our immune receptors or the host biomarkers produced in response. For instance, investigators have used host biomarker signatures generated in response to infecting pathogens as infection signatures. Because the host response to an invading pathogen is naturally amplified, these signatures are easy to measure using simple, user-friendly methods, such as lateral flow immunoassays. Measurement of the pathogen-signatures recognized by our immune system has also led to promising outcomes for early identification of all pathogens early in the pandemic cycle. However, many of the relevant signatures for this approach are lipidated molecules with amphiphilic biochemistry that makes them challenging to manipulate and detect using conventional molecular biology methods (Jakhar et al. 2021; Kubicek-Sutherland et al. 2017). The human immune system is extremely intricate and includes a complicated pattern recognition network. Thus, decoding the complexity of the observed signatures to derive meaningful information is extremely challenging manually. Again, machines can help alleviate this challenge, and mathematical algorithms can greatly increase the usability of these measurements.

Multiomic Approaches

Development of multiomic strategies for the discovery, detection, and characterization of disparate biochemical signatures can greatly enhance our preparedness against invading pathogens. Proteomics and transcriptomics measurements are relatively well developed, and omics measurements and their associated host responses have been used for pathogen characterization and understanding the sequence of events in response to infection. These signatures are viable targets both for diagnostics and for informing treatment options. Lipidomics and metabolomics for diagnostic applications are less developed, both in methods and in associated bioinformatic pipelines and analytics. The significance of lipidomic and metabolomic signatures in human physiology and immunity suggests that these would be valuable areas for more development and investigation (Wang et al. 2019; Kerr et al. 2020).

Genotype to Phenotype Predictions

As mentioned previously, inferring pathogenic properties from novel sequences or complex metagenomic samples is challenging. One emerging approach in this area is the identification of antibiotic resistance markers and virulence genes present in metagenomic samples, without specifically associating individual genes or functions to individual microbes. This approach enables identification of functional potential in environmental samples. For example, the presence of genes or functions that, if moved to the context of a new host organism, could prove problematic. Indeed, the assessment of phenotypic traits can point to genotypic variants of concern in certain pathogens, indicating potential for zoonosis or variations in severity of associated disease (Kubicek-Sutherland et al. 2021; Bush et al. 2016). Taking a different approach, the U.S. Department of Defense's (DoD) Defense Advanced

Research Project Agency (DARPA) Friend-or-Foe program seeks to develop high-throughput phenotypic characterization of a wide array of organisms, thereby improving the ability to predict function or phenotype of organisms bearing novel genes (DARPA 2018; PNNL 2020).

Wearable Technologies

Wearable technologies have also become a wave of the future. Recent wearable technologies can provide information on general health (e.g., heart rate, temperature, activity levels, etc.), making them more effective nonspecific "sickness sensors" for early warning. The array of signatures that can be added to such wearable technologies can be greatly enhanced to include targeted detection (Dunn et al. 2018; Mishra et al. 2020; Vergun 2020). For example, wearables with integrated insulin measurements make real-time diabetes tracking possible, so monitoring disease status is a not-so-distant possibility (Funtanilla et al. 2019). Broad-use development of wearables for disease detection and tracking is in its infancy, but wearables could represent a new pathway for biological detection. Some essential developments needed to expand the application of wearables for monitoring biological threat exposure or infection include: (1) miniaturization of sensors, (2) stable and reversible affinity reagents for longer term monitoring, (3) nanomaterials and sensor designs to enable faster and more sensitive detection, (4) robust and flexible electrical systems, (5) integrated microscale power and data storage, (6) innovative and safe materials for use in such systems, (7) data integration and analysis pipelines, and (8) transdermal biological fluid extraction methods (e.g., microneedles and sweat inducers).

Developing a foundational understanding of exposure and disease biomarkers is another critical need. Notably, data from wearables provide a detailed baseline for an individual rather than a population, offering the potential to detect anomalies with high sensitivity for that person. Yet, cumulative data aggregation power from wearable technologies can provide information about disease progression at the community level. Indeed, as with the current pandemic, early detection, effective disease tracking, and readily available diagnostics to inform actions and therapeutic interventions can greatly help curb the spread. Wearable technologies provide an easily deployed, integrated, and readily assimilated data source. To this end, wearables and other widely distributed sensors represent a major opportunity if they can be integrated into pandemic response pipelines.

Biosurveillance

The needs for biosurveillance system components are similar, but not identical, to biodetection and diagnostics capabilities. Effective biosurveillance requires an architecture that includes multiple data streams and measurements from different sample types. It also needs detection systems that can be used to provide early, community-level identification of emerging threats and their distribution—either through specific detection or through detection of anomalies or indicators of potential biological events (CDC 2012). Biosurveillance can be accomplished by aggregating sensitive results from detection or diagnostic tests on individual samples or by deploying systems to monitor pooled samples (e.g., human, environmental, wastewater, etc.). Thus, depending on the samples and data stream(s), biosurveillance results indicating potential biological events can initiate additional sampling, measurements, or analysis to collect more information to confirm or characterize a biological event and determine appropriate actions to respond and mitigate threat impacts. One example biosurveillance system, BioWatch, uses a panel of targeted qPCR assays to detect aerosolized biological threats (NAM and NRC 2011), making it limited to a selection of pathogens. Therefore, it would be costly to expand this system to cover broader populations and geographical areas.

With regards to untargeted systems, methods based on innate immune recognition can allow us to develop a more broad-based approach for early identification of emerging and hitherto unknown threats. A new bioinformatics pipeline called FEVER (Fast Evaluation of Viral Emerging Risks) allows for the identification of virus families, rather than specific identification of a known serotype or strain (Stromberg et al. 2021). Biosurveillance can also be used to monitor an ongoing biological event over time. For example, during the COVID-19 pandemic, wastewater surveillance is being

used to investigate the distribution of viral variants and their persistence in communities over time (Fontenele et al. 2021). Sequencing of samples (clinical and environmental) for the purpose of biosurveillance would be expensive, but highly specific.

Surveillance tools should be deployed in concert with predictive models and forecasting systems (see Fig. D1, p. 49). Integration of these systems plays a critical role in response efficiency. Various approaches for forecasting infectious diseases exist, but retrospective assessments of any one method's efficacy and reliability pose difficulties. For instance, based on the DARPA Chikungunya challenge (Del Valle et al. 2018) and the CDC's annual influenza forecasting challenges, more robust modeling is clearly required to effectively assess emerging threats via forecasting. Integrating mathematical forecasting with empirical surveillance can greatly enhance outcome reliability, especially if broad-based technologies like the FEVER approach are used.

Crosscutting and Enabling Technologies

The next section discusses two examples of crosscutting and enabling research areas that have the potential to transform biological detection, especially when combined with developments in sensing, engineering, data analysis, and other areas. Advances in materials (including nanomaterials) are also important for biodetection, but that area will be discussed in Section D.5: Materials and Manufacturing, p. 80.

Synthetic Biology

Synthetic biology involves redesigning organisms for useful purposes by engineering them to have new abilities. It merges capabilities in computational design, DNA and biomolecule synthesis, parallelization, and advanced genetic editing to create a toolkit that can potentially accelerate biodetection research. Indeed, synthetic biology has enabled advances in nucleic acid sequencing and affinity reagent engineering (including proteins, aptamers, antibodies (phage/yeast display), and it has been used to engineer phage for the detection of viable pathogens (Sharp et al. 2016). For instance, cell-free RNA logic gates have been developed for virus detection on paper-based biosensors, with colorimetric readouts, making it a simple system that is easy to produce and store. However, such approaches are extremely pathogen-specific and have limited sensitivity. These challenges should be addressed before potential use of these approaches in biodetection architectures.

Synthetic biology approaches have also been used to engineer living cells for sensing. Synthetic biology can enable cell-based detection for identification of intracellular molecules and processes. These include monitoring cellular response to threats, target specific transcription (e.g., resulting in the production of fluorescent proteins), and riboswitches (Sanbonmatsu 2014). While cell-based sensing provides some benefits (e.g., relatively inexpensive), broad application of these methods is challenging, owing to difficulties in coupling to monitoring systems for readout, the need to maintain cell viability, and relatively slow response times of minutes to hours (rather than seconds). Still, understanding these intracellular signatures and events can enable accelerated diagnostic and therapeutic development in the event of an outbreak.

Single Pathogen Analysis

Direct analysis of single pathogens is potentially transformational for rapid, sensitive detection. Currently, qPCR can provide detection down to the limit of single pathogens but requires a time-consuming enzymatic amplification process. Current laboratory-based systems can detect single cells and subcellular components, but they are generally too expensive and complex for routine or large-scale use. For example, super-resolution fluorescence can be used to quantify proteins and nucleic acids (Shay et al. 2016); single-cell nucleic acid sequencing technologies have been developed (Hwang et al. 2018); and methods for single-cell proteomics are emerging (Perkel 2021; Yeh et al. 2010). However, advances in analytical instrumentation are needed to develop lower cost and simpler analytical approaches at the single-cell and subcellular levels for application beyond laboratory research. In addition, innovative sample preparation will be critical for practical use of such technologies. To that end, droplet-based microfluidics approaches can be used for processing and manipulating small sample volumes at the single-cell level. However, routine, low-cost detection with single pathogen sensitivity, especially in real-world samples, will require further research and innovation.

D2.2 Needs for Strengthening Biopreparedness: Surveillance, Detection, and Diagnostics

Requirements Development

Understanding the differences in requirements between specific diagnostics, environmental detection, and broad-based surveillance is critical to inform foundational and applied research needs. While these capabilities share many similarities, they are also marked by differences. For instance, surveillance methods should be capable of identifying emerging threats, be easily deployable at large community scales, and be associated with data integration and systematic response strategies. This last capability can include additional sampling, data collection, and analysis to gain more information if emerging biological events are suspected. On the other hand, diagnostics should be individually tailored and accurately analyze complex human samples, with the results tied to individual actions and therapeutic intervention. The requirements of these detection systems can be different, and an in-depth investigation and assessment of these needs can facilitate targeted research and development. In addition, all detection and diagnostics systems should consider the whole system—from sample to answer—to develop integrated sample preparation and detection approaches to meet needs. Also, as previously noted, significant synergy exists across requirements for countering biological threats (whether natural, accidental, or intentional), warfighter support, global public health, and pandemic preparedness. Understanding these synergies and promoting interagency coordination can also help us achieve our goals faster and with minimal redundancy.

Pipeline Development: From R&D to Solutions

Preparation for the next pandemic requires an integrated pipeline that addresses key requirements from foundational research and innovation to solutions for deployment. In areas of surveillance, detection, and diagnostics for pandemic preparedness, this involves the integration of microbiology and assay development with the fields of engineering, data science, materials sciences, chemistry, and physics, to name a few. This also requires partnerships and collaborations across federal departments, academe, industry, and end users. Rather than working in isolation, collaboration and continual feedback is needed between foundational research, applied research, and end users to understand research needs and facilitate the innovation that is needed to develop surveillance, detection, and diagnostics solutions.

Pathogen-Agnostic Detection Strategies

Diagnostics and detection systems are still largely pathogen-specific. Such methods are extremely valuable for addressing specific targets, but they do not prepare us for the next major threat looming on the horizon. The development of rapidly deployable and usable pathogen-agnostic strategies can greatly advance our pandemic preparedness. These could include sequenc ing technologies and the development of other analytical methods and instrumentation. Developing new pathogen agnostic detection approaches and expanding on current capabilities, including further development of spectroscopy and spectrometry to include biological targets, could revolutionize our preparedness stature.

Additional Surveillance Data Streams from a One Health Perspective

Opportunities exist to further improve surveillance and forecasts of emerging threats by integrating data streams with information about pathogens that affect animals and plants and those present in the environment. Approximately 75% of new and emerging diseases are zoonotic in origin, and animals provide additional reservoirs for pathogen evolution, impacting the progression of an outbreak or pandemic. Environmental factors also affect pathogen spread, thereby influencing health outcomes. Environmental change significantly affects pathogen transmission, whether through air, water, food, or vectors, such as mosquitoes (Bartlow et al. 2019). Modular surveillance and forecasting approaches are needed for integrating new data streams to evaluate their usefulness for improving the understanding of pathogens and their variants. Additional evaluations of these integrated models and forecasts for their abilities to provide early warnings and to inform response are also needed.

Additional Technology Needs

It is also important to understand specific limitations and needs for each of the current detection, diagnostics, and surveillance approaches outlined in the Section D2.1 in relation to requirements for the application of the technology. In many cases, additional R&D is needed to enable lower cost, faster, higher confidence detection in real-world samples. For example, with sequencing-based technologies, R&D is needed to (1) understand the background of biosignatures and effectively identify pathogens among normal microbiota; (2) determine appropriate samples for analysis; (3) optimize sample processing and data analysis; and (4) develop innovative, lower cost, rapid sequencing approaches for detection, diagnostics, and surveillance applications. With spectroscopy and spectrometry, integration of the technologies with advanced data analysis and AI/ML approaches can greatly enhance our ability to understand and interpret data. Understanding how to expand on personalized medicine and wearable technologies with all detection approaches is also important, as this data could potentially be tapped and utilized in the event of an emergency. Many other needs for strengthening biopreparedness are highlighted during the discussion of each technology area in Section D2.1.

Biological Sample Handling

Another important need for biopreparedness is having appropriate biocontainment levels available to work with pathogens. While surrogates or near neighbors to pathogens can be used during some phases of R&D, experimental research with pathogens (often requiring biosafety level 3 [BSL-3] containment) is necessary for activities such as developing and testing affinity reagents; developing and optimizing sensing and detection approaches; and testing the performance of detection, diagnostics, and surveillance systems.

D3. Molecular Mechanisms, Systems Biology, and Molecular Therapeutics

In responding to a pandemic threat from a novel pathogen, molecular therapeutics need to be rapidly and efficiently developed and distributed to prevent the pathogen from spreading. Developing molecular therapeutics for combating emerging pandemics requires a fundamental and comprehensive understanding of how a pathogen infects a host, replicates and assembles itself, and evades the host immune system. Elucidating these mechanisms requires advanced instrumentation and techniques for characterizing the molecular-level structure and dynamics of pathogen-host interactions, along with improved multiscale experiments that enable a systems-level analysis of functional aspects of these interactions (see Fig. D2, this page).

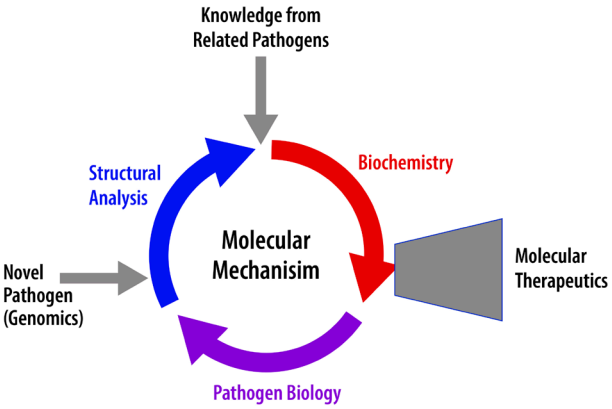

Fig. D2. A predictive understanding of molecular mechanisms, required for developing molecular therapeutics, is built on determining the function(s) of each protein, and how each protein carries out those function(s).
[Image credit: Lawrence Berkeley National Laboratory]

A strategic approach that leverages DOE capabilities could be used to accelerate the molecular therapeutic developmental pipeline. Starting from identification and genetic characterization of the pathogen, scientists can isolate component proteins, solve their structures, and characterize their mechanisms, enabling prioritization of potential lead therapeutics. Further, systems-level data and subsequent modeling of host response provide insights into the mechanisms of pathogenesis and help determine targets for therapeutic development and efficacy of lead therapeutics. With scientific expertise and facilities in genomics, structural biology, and computing, DOE provides critical support for molecular therapeutics research efforts in academe, government, and industry. Importantly, DOE synchrotron X-ray and neutron sources provide high-resolution protein structural information, which

can be used to help identify potential molecular therapeutic targets and to determine potential small molecule therapeutics *in silico*.

In this section, we describe the current state of the art in the three interrelated areas of molecular mechanisms, systems biology, and molecular therapeutics; and we also outline needs for strengthening these areas to address future pandemic threats.

D3.1 Current Capabilities and State of the Art: Molecular Mechanisms

An effective molecular therapeutic will target vulnerable mechanisms of the pathogen-host infection cycle or directly compromise the viability of the pathogen to prevent continued persistence in the host and infection of other individuals. Molecular mechanisms include all parts of the infectious cycle—binding and entry to the host, establishment of the pathogen in host cells, immune evasion, production of pathogen proteins and replication, assembly, and egress from the host (see Fig. D3, this page). This mechanistic knowledge, based on genomics, structural biology, biochemistry, and virus and bacteria biology, is critical to understand pathogenesis, the systems biology of host responses, and pathogen evolution and underpins the identification and design of target molecular therapeutics. Recent technological advances have allowed unprecedented gains in our knowledge of the molecular mechanisms of many different pathogens of interest, including, for example, influenza virus (te Velthuis and Fodor 2016; Yamauchi 2020; Dawson et al. 2020).

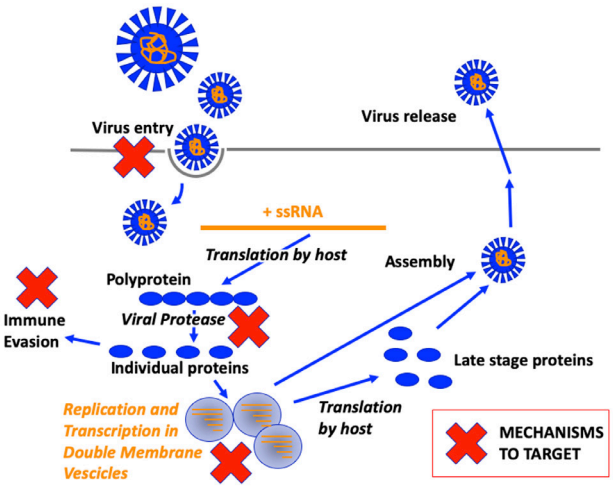

Fig. D3. Schematic of SARS-CoV-2 life cycle with mechanisms strategic to target for molecular therapeutics.
[Image credit: Lawrence Berkeley National Laboratory]

Bioinformatics for Protein Function Prediction

Determination of genome sequences is the starting point for structural and functional analysis of pathogen proteins. Protein sequences are essential for determining or predicting structure and thus identifying potential drug targets. Methods to examine, for example, evolutionarily conserved regions of proteins are used to identify potential ligand binding sites and interfaces that in turn can be used to prioritize targets. Additionally, sequence and domain similarities can help researchers predict functions. One example is SARS-CoV-2 work on essential proteins for cell entry, cell replication, and immune evasion, which was jumpstarted by research on other coronaviruses. For novel pathogens, these approaches may be limited by lack of similarity with known functional domains or by poorly understood multifunctionality of individual proteins. For SARS-CoV-2, bioinformatics analyses combined with structural analyses identified critical active sites and interfaces in SARS-CoV-2 proteins and may play a larger role in determining mechanisms in novel pathogens. Finally, evolutionary sequence analysis can provide insight into the potential variation paths a pathogen might take in its evolution. Bioinformatic analyses of sequence information arising from real-time monitoring of virus evolution can also be important for developing vaccines and identifying resistance to therapeutics.

Production of Pathogen Proteins

Once the genome sequence of a pathogen is determined, it is possible to produce recombinant proteins needed for structure, biochemistry, and drug discovery critical for the development of molecular therapeutics. In most cases, recombinant expression in bacteria is efficient and was useful for producing many SARS-CoV-2 proteins. However, certain viral proteins require expression in eukaryotic cells due to the need for chaperone proteins, rare codon usage, and post-translational modification (Wang, B., et al. 2021). Membrane proteins also require special consideration

regarding host expression choices or the use of scaffold systems for supporting solubility and stability on a native-like lipid (Ritchie et al. 2009). Furthermore, for proteins that have flexible or disordered regions, targeted or random mutagenesis may be needed to steer the protein to a more stable conformation and increase protein production. Stabilization of the spike protein was critical for successful SARS-CoV-2 structural studies and facilitated development of RNA vaccines that use this mutant sequence (Schaub et al. 2021). Some viruses (such as retroviruses, coronaviruses, and flaviviruses) produce multiple proteins as a single polyprotein that is post-translationally cleaved by viral or cellular proteases (Yost and Marcotrigiano 2013), resulting in multiple individual proteins. Once a protein is obtained, biochemical and structural analyses can be initiated in parallel.

Biochemical Analysis to Validate Mechanisms

A critical component in designing molecular therapeutics is the development of assays to test potential bioactive compounds identified from computational screens and to test mechanistic hypotheses. Generally, assays are either biochemical or cell based (Hughes et al. 2011). Given their scalability, throughput, and mechanistic relevance, biochemical assays are typically the first type of assay employed. *In vitro* biochemical characterization requires a variety of assays that probe the functionalities of diverse classes of proteins (e.g., polymerases and proteases) and are both sensitive and specific to the target(s) of interest. Such assays are commonly based on fluorescent or colorimetric sensors of target protein activity, although label-free techniques for protein-protein and protein–small molecule interactions are also increasingly utilized in drug discovery. Assay robustness can vary, and some may not readily translate to all target proteins of a given functional class. Furthermore, novel targets can require extensive assay development where standardized, validated assays are lacking. Regardless of target or assay format, key considerations in assay development include (1) reliable performance, (2) quantitative output with broad dynamic range, (3) relative simplicity, and (4) predictive value. Although biochemical assays afford efficient, cost-effective screening at scale, the contrived nature of such assays limits translatability and motivates the follow-on execution of cell-based assays as a next step in candidate drug characterization.

Cell Biology, Organoid, and Animal Analysis

Cell-based experimentation is indispensable in therapeutics discovery and development, particularly in the initial identification of druggable targets and the assessment of candidate drug function in an *in vivo* context. Such assays are generally more complex and less scalable than *in vitro* biochemical assays, but they are typically more biologically relevant and therefore better able to predict outcomes in animal and human experiments (Hughes et al. 2011). Cell-based experiments presuppose an ability to propagate pathogens and target host cells (or suitable proxies), a nontrivial caveat particularly for emerging and fastidious pathogens. Additionally, the intrinsic risk associated with high biosafety level (BSL) pathogens requires special hazard protocols in BSL-3 or -4 laboratories, the relative paucity of which hindered SARS-CoV-2 work and created barriers to the rapid testing of mechanistic hypotheses and development of therapeutics (Yeh et al. 2021). However, elegant workarounds may be employed to reduce dependence on specialized infrastructure, including the use of pseudoviral chimeras (Syed et al. 2021), attenuated derivatives, or relatives of target pathogens that only infect mammals other than humans (Hackbart et al. 2020).

Unfortunately, conventional cell-based assays sometimes fail to reflect the relevant biology and may yield artifactual results due to the difficulty in recapitulating the complex multicellular interactions found in natural tissues through laboratory cultivation. Recent advances in the development of organotypic three-dimensional (3D) culture models have led to the development of cell-based systems that more faithfully reflect the physiology and environment of relevant cell types in *ex vivo* models (Langhans 2018). Such models have served as efficient, tractable alternatives to tissue explants or animal models in the translational study of enteric or respiratory infections, specifically in investigating the pathophysiology of SARS-CoV-2 during human infection (Youhanna et al. 2021). 3D cell culture and organoid models have been increasingly deployed in various stages of drug discovery and development, including pharmacokinetics, drug

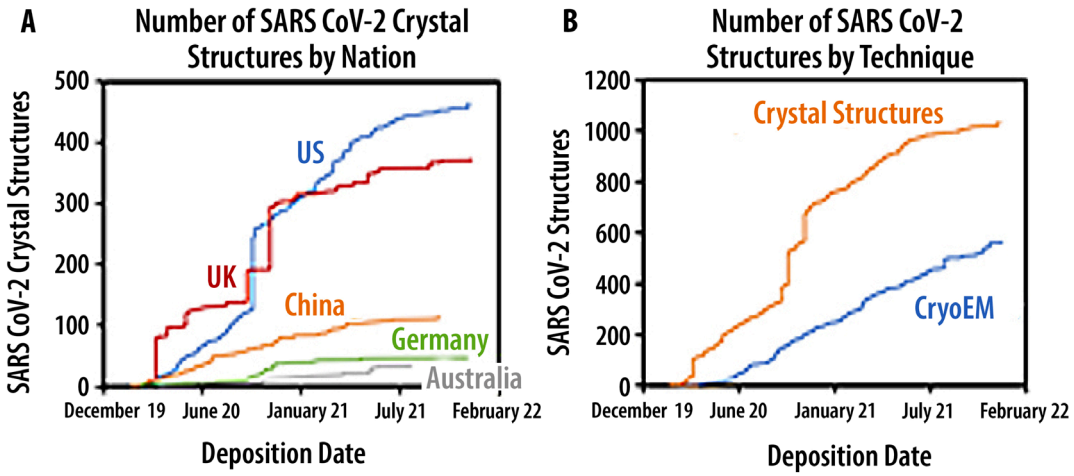

Fig. D4. Timeline for structure deposition of SARS-CoV-2 proteins.
[Image credit: Lawrence Berkeley National Laboratory]

metabolism, and toxicity analysis (Zscheppang et al. 2018; Shen et al. 2020).

Despite advances in *ex vivo* models, animal studies remain the gold standard for systems-level interrogation of pathogenesis and represent an essential component of any drug discovery campaign (Takayama 2020). Effective model selection is critical; model organisms that are highly divergent from humans (e.g., rodents) exhibit variable relevance to human disease depending on the indication, but primate studies, while generally more predictive, require specialized expertise and facilities, not to mention significant expense. The utility of animal models in development of therapeutics targeting infectious diseases includes species-dependent variation in innate and adaptive immunity, structural divergence in receptors required for viral entry, and distinct commensal microbiota that can influence the immune response (Colby et al. 2017). Careful consideration of which animal models, including alternative ones, to use for a given pathogen are important going forward to ensure best use of limited resources.

Structural Analysis by X-Ray Crystallography, Cryo-Electron Microscopy, and Solution Techniques

Structures are essential for visualizing the molecular machinery of viral mechanisms, therapeutic development (see Fig. D3, p. 60), and interpretation of data from systems biology approaches (see next section).

In response to COVID-19, structural biologists in academia, government, and industry rushed to structurally analyze SARS-CoV-2 proteins. They used all tools available, including DOE synchrotron and neutron sources and cryo-electron microscopy (cryo-EM) facilities. Many high-resolution structures were deposited into publicly available databases that other scientists were then able to access to probe mechanisms and inform drug discovery efforts. As of January 2022, the atomic structures of almost all SARS-CoV-2 protein domains (41 of 47) have been structurally determined (NCBI 2022) and released to the global scientific community, representing a massive scientific effort. The rapid contribution of DOE X-ray facilities to the pandemic response, relative to other nations, is shown in Fig. D4, this page, which represents only structures deposited in publicly available databases. As industry researchers typically do not deposit their structures, these numbers are under-represented. Viral protein structures revealed active sites, along with protein-protein and protein-RNA interaction interfaces, essential for mechanistic understanding. For biopreparedness, the rate at which we can resolve these structures, across the viral genome, is critical for subsequent scientific effort to develop downstream medical countermeasures.

Dynamical Structure Analysis

To complement these atomic structures, solution methods such as small-angle X-ray scattering (SAXS),

small-angle neutron scattering (SANS), and X-ray foot printing provide structural information on protein conformational flexibility and assembly processes. A collaboration among three user facilities at DOE national laboratories (Lawrence Berkeley, Argonne, and Oak Ridge) combined X-ray crystallography, SAXS, and SANS analysis of the assembly of the SARS-CoV-2 replication-transcription complex (RTC), Nsp12-Nsp7-Nsp8. The collaborative analysis revealed that RTC proteins have multiple assembly states with distinct RNA binding capabilities and provided targets for disrupting replication-transcription (Wilamowski et al. 2021). X-ray foot printing defined spike protein conformational dynamics mechanistically involved in the virus' cell invasion mechanism (Schoof et al. 2020). These studies highlight the importance of a multipronged approach for structural characterization. Additional techniques at DOE facilities were applied in response to COVID-19, including X-ray tomography to characterize virus-infected cells (Loconte et al. 2021) and neutron crystallography to follow protonation in the catalytic mechanism of the main protease (3CLpro; Kneller et al. 2021).

Structure Prediction and Dynamic Simulations

In the past year, a revolutionary advance in protein structure prediction has promised to change structural biology. Alphafold software, which uses attention-based and nonattention-based machine learning (ML), was able to provide accurate protein structure predictions for ~80% of the targets at the 2020 Critical Assessment of protein Structure Prediction (CASP), a community-wide experiment that provides unbiased assessment of prediction algorithms (Kryshtafovych et al. 2021). However, for pathogen biology, these ML approaches still present challenges, including limitations in the training set and the inability to identify the most biologically relevant protein conformations and predict complexes and small-molecule binding.

Once protein structures are available, whether experimentally or computationally determined, computational structural biology using molecular dynamics (MD) can be performed, which has informed mechanistic understanding of SARS-CoV-2 infection (Casalino et al. 2021). MD capabilities include both all-atom and coarse-grained MD simulations, along with graph theory and ML to extract molecular mechanisms. These tools can elucidate structural and functional implications of emerging mutations, identify allosteric effects, address immune evasion, and quantify the molecular recognition of host receptors. Combined with other modalities, such as high-resolution molecular-level imaging, MD capabilities have provided valuable new insight into the mechanisms of host-pathogen interactions (Leigh and Modis 2021).

D3.2 Current Capabilities and State of the Art: Systems Biology

Systems biology can advance our understanding of the complex interplay between host and pathogen and how this interaction causes disease. Systems biology research strives to identify important molecular components of a system and the relationships among these components and then formulates these as computational models that ideally can represent and predict emergent properties arising from the system. Such studies can be used to understand the pathogenesis of a disease at scales from individual cells to human populations and to identify novel targets for therapeutic intervention.

Fundamental Science for Systems Biology

The current state of the art in systems biology for pathogen characterization is based on research-oriented fundamental science programs. As such, there is not a consistent set of approaches, models, or analysis techniques that can be easily compared. This section provides an overview of the types of approaches and data and describes success stories from systems biology interrogations and modeling of host-pathogen interactions. These studies have several outcomes relevant to biopreparedness, including (1) identification of targets for molecular therapeutic development, (2) development of models that can help identify and predict susceptibility to a specific pathogen, and (3) characterization of pathways and molecular mechanisms that are common to pathogenic response or give rise to systems-level phenotypes such as disease symptoms and pathogenesis.

New experimental tools and computational advances have expanded our capabilities to study the systems biology of pathogenesis from infectious diseases

(Eckhardt et al. 2020; Aderem et al. 2011). Much of our knowledge of pathogenic mechanisms derives from classical experimental approaches (Western blot, RT-qPCR), but development of high-throughput technologies such as RNA sequencing, proteomics, and single-cell assays have revolutionized our capabilities to study these processes (Suomalainen and Greber 2021).

Experimental Advances

Common omics approaches include measurement of mRNA expression levels and measurement of protein levels via mass spectrometry–assisted proteomics. Protein modifications like phosphorylation, ubiquitination, and acylation are also used to investigate signaling pathway activity in response to infection and can be important to determine the specific modes of action for pathogens, at least at the cellular level (Stukalov et al. 2021). Other approaches for studying host-pathogen interactions are metabolomics and lipidomics, or the characterization of small molecules and lipid species, respectively, at the molecular level by mass spectrometry (Iqbal and Garrett 2020).

Human and other animal host responses to pathogens involve complicated and varied processes that are contingent on genetic variability and immune response, as well as the responses of individual cell types, tissues, and organs. Thus, many current studies attempt to characterize pathogen interaction with multiple cell types or in multiple genetic backgrounds (Bourgeois et al. 2021). Organoid and animal models provide information at the system-level that can't be gleaned from individual cell culture studies. However, results and data from these experimental models may not directly translate to understanding infection processes, immune response, and pathogenesis in humans.

Beyond measurements of abundance levels for molecular components, understanding the function of biological systems requires knowing about the molecular complexes and pathways involved in cellular and organismal response. Current approaches to characterize biomolecular interactions are based on genetic techniques and proteomics on complexes enriched using a variety of approaches (Baggen et al. 2021). This analysis provides information about the physical interactions between pathogen proteins and host proteins and pathways and, therefore, insight into the functional interactions between pathogen and host at the cellular level.

Another important method for determining critical host components for response to pathogens is functional screening using genetic manipulation such as CRISPR (Bourgeois et al. 2021). In this approach, tools are used to interfere with the expression of individual host proteins in a high-throughput assay; infection success is then evaluated using a phenotypic readout (cell death or other markers of infection). These studies provide a catalog of the host proteins critical for successful infection at the cellular level.

In addition, advanced imaging technologies for viruses (Wang et al. 2018) and bacteria (Kapanidis et al. 2018) generate large datasets that provide insights into the infection process and interactions with the host. Notably, all these new technologies generate more quantitative data than classical assays, but they also introduce additional complexities in data analyses (Chen et al. 2019).

These capabilities have allowed the scientific community to develop detailed knowledge of the life cycle of different viruses, including cell entry, uncoating, genome replication, assembly, and egress from infected cells (see Fig. D3, p. 60). The approaches also have revealed aspects of innate (and adaptive) immune system responses. The level of knowledge (as assessed, for example, by the number of scientific publications) is very heterogeneous across different viruses and has depended mostly on the interest of the scientific community. For example, HIV, hepatitis C, and influenza have been studied in detail, but other viruses only garnered more interest upon outbreaks in the human population (e.g., Zika, SARS, and coronaviruses). Due to biocontainment level requirements, other viruses are even more difficult to study, including Ebola and Nipah viruses, which require BSL-4 containment and can be studied only by a handful of experimental laboratories.

Modeling Host-Pathogen Relationships

Systems models of host-pathogen interactions and outcomes take several different forms depending on the objective of the study. Often the relationship of

the host and pathogen and the response of the host are formulated as a network of interacting components (e.g., transcripts, genes, metabolites, and other molecules). This formulation has several advantages such as intuitive interpretation, simplicity, and ease of integrating different types of information. Networks can be constructed directly from data (i.e., interactomics, as discussed above), by interpreting data in the context of known interactions and pathways, and by inference of relationships using statistical methods or ML (Eckhardt et al. 2020). These kinds of models can be used to identify critical host nodes for pathogen response, examine pathway activation in response to infection, and focus on sets of components that can serve as biosignatures of pathogenesis and susceptibility (Eckhardt et al. 2020; McDermott et al. 2016).

A primary goal of systems biology modeling is to better understand the molecular interactions with the host and to identify those interactions that might be good targets for developing molecular therapeutics. Though the use of host targets for therapeutic development has not been extremely successful, it still represents an important potential for novel therapeutics that could provide, for example, efficacy across a broad pathogen range. Insights into molecular interactions between the host and pathogen are also important for understanding potential unintended off-target effects as well as the impact such therapeutics might have on system-wide outcomes, such as immune response and pathogenesis. A deep understanding of system-wide outcomes will likely require integrating systems biology and more targeted mechanistic approaches (Arazi et al. 2013).

To extend beyond the cellular and molecular levels, researchers have developed models of human immune system dynamics and molecular- to organ-level models for infectious disease (Handel et al. 2020; Wang, S., et al. 2021). These models often target specific aspects of infection and host response; challenges remain for developing approaches that integrate systems-level molecular measurements and knowledge from human disease and other systems.

Mechanistic modeling is limited in terms of scale and applicability to agents with unknown pathogenesis. Advances in artificial intelligence (AI) and ML have been applied to analyze host-pathogen systems biology data to better identify patterns and pathways from multiomics data (Carapito et al. 2022). Increasingly, high-performance computing (HPC) capabilities are required for these efforts and computation to parameterize complex models of infection and pathogenesis.

D3.3 Current Capabilities and State of the Art: Molecular Therapeutics

When a new pathogen emerges, the interval from first human infection to declaration of a global pandemic can be as short as several weeks. It is therefore critical to have a robust and rapid therapeutics discovery, development, and manufacturing pipeline, as exemplified during the COVID-19 pandemic. In preparation for the next pandemic, a broad range of therapeutic classes should be considered, including small molecules, proteins (e.g., antibodies or interferon-based agents), cell-based therapies, engineered microorganisms, and autologous transfer (e.g., platelet-rich plasma). This document focuses on molecular therapeutics, which include many disparate kinds of molecules such as small-molecule inhibitors, peptide-based inhibitors, nucleotides, monoclonal antibodies, self-replicating RNA, and viral interfering particles (Meganck and Baric 2021). In general, molecular therapeutics design follows a similar process used for all therapeutics and vaccines, including design, testing, development, and manufacture. However, each type of therapeutic has bottlenecks in the process that need to be overcome to shorten the time between design and market.

The type of molecular therapy to develop may be dictated by the mechanism being targeted (Bhatti et al. 2020). Common viral and bacterial mechanisms include entry, protein processing and synthesis, and replication (see Fig. D3, p. 60). Other target mechanisms may involve various host factors such as membrane-bound receptors (Chitalia and Munwar 2020). Knowing the exact mechanisms to target is essential for an inhibitor design strategy. When targeting viral entry, for example, common therapies include viral interfering particles (decoys for cell adhesion), monoclonal antibodies or nanobodies (inhibition of target cell interactions), small molecules (e.g., endocytosis or fusion inhibitors), and peptide-based therapies (viral envelope inhibitors; Tuccori et al. 2021; Gupta et al. 2021; Xu et al. 2021). For targeting protein processing

or synthesis, the most common therapies are small molecules (protease inhibitors, Chia et al. 2021); and for targeting replication, nucleotide or nucleotide analogs are often used (Götte 2021). When targeting host cells, a general strategy is to interfere with cellular membrane composition or biophysical properties using small molecules to limit, for example, viral fusion to cells (Plavec et al. 2021). Although targeting specific cellular proteins or receptors is also possible, there is a risk of severe side effects (Margolis and Archin 2017). For biopreparedness, the ideal molecular therapy is broad spectrum or broadly neutralizing, which may require targeting a universal mechanism. In addition to targeting many pathogens at once, having broad efficacy may also stifle emergent variants or drug resistance. If this strategy is not possible, then many therapies in combination will be needed for broad efficacy and controlling rampant spread.

Compound Screening and Design

In addition to the type of therapy matching the molecular mechanism, a fast, efficient suite of assays to screen candidate compounds against the targeted mechanism is critical for successful drug design. The Molecular Mechanism and Systems Biology sections present the current state of the art for identifying druggable mechanisms. Though many mechanisms may be possible for molecular therapeutic intervention, proteins with the most reliable assays are likely to become identified protein targets for molecular therapeutics because experimental testing is needed to verify binding to the protein targets.

The first step in molecular therapy design is screening compound libraries to find an initial "hit" molecule. Many drugs on the market have been designed using a phenotypic screening approach. In this case, the experimental assay suite is the key to the design-synthesize-test iterative cycle to advance the design of the candidate molecule because no structural information is available. The complementary essential partner for successful phenotypic screening is access to large chemical libraries. Nevertheless, virtual ligand screening is still possible with quantitative structure activity relationship analysis.

Structural biology is an essential capability for identifying hits and optimizing lead compounds to become molecular therapeutics. Structural biology can reveal active sites and molecular mechanisms as described earlier in this section and serves as the basis for using a structure-based drug design (SBDD) approach. X-ray crystallography remains the best technique for experimentally determining how a ligand is bound within a protein, followed by cryo-EM. For SARS-CoV-2, there were more than 1,000 crystal structures deposited of proteins bound to ligands and almost 500 cryo-EM structures. Crystal and cryo-EM structures were critical for development of COVID-19 molecular therapeutics, including Monuparavir (Kabinger et al. 2021) with Nsp12-Nsp7-Nsp8, VEKLURY® (Remdesivir) with Nsp12-Nsp7-Nsp8 (Kokic et al. 2021; Wang, Q., et al. 2020; Bravo et al. 2021), and PAXLOVID™ (PF-07321332) with 3CLpro (Zhao et al. 2021; Owen et al. 2021; see Fig. D5, this page). We expect that more inhibitors are forthcoming, including those developed through DOE's National Virtual Biotechnology Laboratory (NVBL). Although predicted protein structures have been used for drug discovery and are promising alternatives for unknown proteins, they have overly perfect geometries, lack bound critical water molecules, and can lack other details needed for drug design. Still, available structures facilitate using

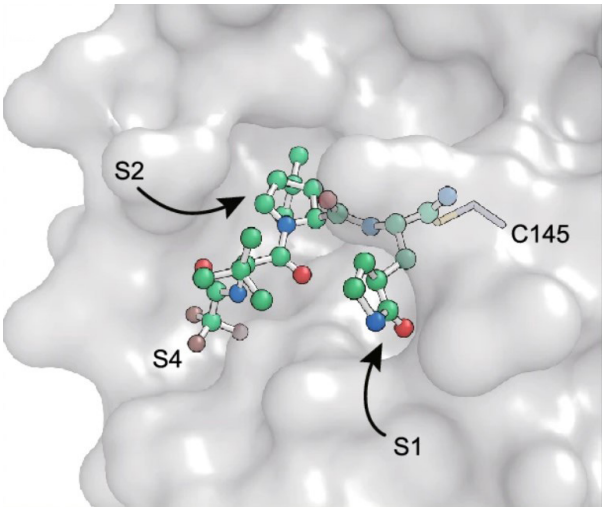

Fig. D5. An example crystal structure of a ligand bound in the binding site of the SARS-CoV-2 3CLpro protein.

[Image credit: Reprinted under a Creative Commons Attribution 4.0 International License (CC BY 4.0) from Zhao, Y., et al. 2021. "Crystal Structure of SARS-CoV-2 Main Protease in Complex with Protease Inhibitor PF-07321332," *Protein & Cell*. DOI: 10.1007/s13238-021-00883-2.]

virtual screening methods prior to experimental assay to prioritize the compounds to be tested in order to increase the experimental success rate for finding tighter binding compounds.

Computational Compound Screening

Virtual screening is a computational technique in drug discovery that searches compound libraries for small molecules that bind to a given protein target (Rester 2008; Schneider et al. 2019). Ultra-high-throughput virtual screening (uHTVS) is an effort to expand small compound libraries to include well beyond 100 million compounds and eventually more than 15.5 billion compounds (Johnson and Karanicolas 2016). Virtual screening is classified into three subtypes: ligand based, structure based, and hybrid methods (McInnes 2007). Ligand-based screening focuses on comparing sets of active and decoy ligands to develop a ligand-centered search criterion to filter small-molecule compound libraries. Examples include pharmacophore searches (comparing pharmacophores of active compounds to a library to assess goodness), similarity searches, or even property screens using algorithms or ML models (Joshi and Kumar 2021; Joshi et al. 2021; Yan et al. 2016; Lin et al. 2020; Horvath 2011; Sun 2008; Kumar and Zhang 2018; Coley et al. 2017). Structure-based molecular docking requires a protein structure and a scoring function to assess the most likely 3D ligand position within a protein binding pocket, referred to as a pose. Traditionally, these approaches have used physics-based methods. Hybrid methods combine the two practices into a single pipeline. Current uHTVS techniques have largely focused on leveraging cloud computing (Gorgulla et al. 2021; Chodera et al. 2020) or DOE's or the National Science Foundation's supercomputing infrastructure (e.g., AMPL) to discover viable hits for various biomolecular targets (Lee et al. 2021; Acharya et al. 2020; Clyde et al. 2022; Stevenson et al. 2021; Jacobs et al. 2021; Jones et al. 2021; Hinkson et al. 2020; Minnich et al. 2020). Recent developments in AI/ML approaches have been pushing the boundaries on the accuracy and speed of discovering true positives from large datasets (and filtering out false negatives) using a variety of techniques including graph neural networks and reinforcement learning–based ideas (Kimber et al. 2021; Chong et al. 2021; Goel et al. 2021).

Synthesis and Lead Optimization

The most significant recent advances in synthesis are the variety of combinatorial chemistry methods that can generate extremely large and diverse chemical libraries (>1 million) that are frequently focused by chemistry and reagents, such as phage-display, bacteria-display, yeast-display, mRNA-display, DNA-encoded chemical libraries, and one-bead-one-compound libraries (Liu et al. 2017). These compounds are ideal for phenotypic screening and are now used for hits to optimize lead compounds. Many drug development programs still use traditional single-molecule synthesis when modifying specific functional groups to optimize lead compounds and make them more druggable.

The iterative process to optimize hits to become lead compounds and ultimately druggable candidate therapeutics utilizes a variety of methods to improve the design. For phenotypic design, more assays are added to validate every aspect of the desired outcome. For a SBDD approach, experimental structure determination of ligand-protein complexes is essential and can guide added functionality (e.g., H-bond acceptor/donor) to favor interactions with the protein and remove noninteracting functionality so that the inhibitor remains in the drug-like chemical space. To boost potency, functionalities can be designed to displace or take advantage of ordered water in structures (Wong and Lightstone 2011). For this activity, high-resolution structural biology is critically important to show the exact geometry of chemical groups in the active site, as well as location of ordered waters. Thus, the complexes can suggest new designs to synthesize.

As compounds become more efficient binders to their protein targets (typically <10 nmol), druggability is tested by adding more experimental assays, such as solubility, stability, plasma binding, hERG inhibition, permeability, and clearance. Optimization of compounds is guided by experimental results, from simple physicochemical properties to specific cellular behavior to tissue-specific outcomes. The general belief is that each experiment helps identify compounds that fail to meet a druggable property and are then sent back for redesign. During these development stages, drug

absorption, distribution, metabolism, and excretion (ADME) properties are predicted by additional computational and experimental assays and rodent animal studies (Alqahtani 2017). When sufficient druggable properties are met, higher-level animal studies are performed to test near-human organism phenotypes, hoping they will be predictive of human results. A full description of experimental testing is provided in the Molecular Mechanisms section.

Biologics

The coming of age of biologics—biological molecules used as molecular therapeutics—was demonstrated in the COVID-19 pandemic, and indeed, biologics were the earliest molecular therapeutics developed specifically for SARS-CoV-2, as famously exemplified in the treatment of the President of the United States by Regeneron's REGN-COV2. Many COVID-19 biologics are based on antibodies or nanobodies (smaller versions of antibodies) against the SARS-CoV-2 spike protein. These neutralizing antibodies or nanobodies are designed to interfere with the initial entry of the virus into the cell and with its spread within infected individuals (Cherkasov et al. 2009; Mohanty and Mohanty 2021; Narayanan et al. 2021; Schneider et al. 2020). Their antigen sites on the spike protein were determined by X-ray crystallography and cryo-EM, underscoring the role of DOE facilities and structural biology in the development of biologic-types of molecular therapeutics.

Rapid Multimodal Drug Delivery Platforms

Optimization of drug formulation and delivery is needed for any therapeutic. Recent advances in nanoparticle technology have opened a new method of drug delivery. However, the inability to target drug-filled nanoparticles to virus-infected tissue-specific sites has been a limitation. A new rapid multimodal technology under development uses nanoparticles, such as liposomes, with SARS-CoV-2 viral spike protein embedded on the surface to target human cells most likely to encounter the virus. Inside the nanoparticle is the therapeutic agent, such as protein, mRNA, small molecule, or CRISPR system, creating a targeted delivery system. The nanoparticle can be thought of as a type of UPS truck for the human body, and the spike protein is the zip code where the therapeutic agent needs to be delivered. This approach enhances efficacy and reduces toxicity of the drug by delivering the therapeutic agent only to cells that can be infected by SARS-CoV-2. This platform is generally applicable to many virus families and could, when integrated with high-performance *in silico* drug discovery, enable rapid responses to biological events.

D3.4 Needs for Strengthening Biopreparedness: Molecular Mechanisms, Systems Biology, and Molecular Therapeutics

Biological Sample Handling

An important need for pandemic preparedness is to have appropriate biocontainment levels to work with infectious agents, particularly new pathogens whose initially established biosafety level requirements are typically higher than well-characterized pathogens due to their unknown characteristics. It is crucial to have appropriate biosafety facilities capable of high-throughput genomics (i.e., next-generation sequencing), *in vitro* infection experiments (including development of 3D organ cultures), or high-throughput infection capabilities, such as organoids on a chip.

Protein Production and Analysis

Currently, the bulk of methods for determining molecular mechanisms are low throughput. They can require significant biochemistry knowledge and supporting disciplines in molecular, cellular, and microbiology. Most biochemical assays also rely on purified enzymes that tend to be highly engineered for solubility. Better methods are needed to access both pathogen and host membrane-bound proteins for assay development. There is also a need for improved quantitative platforms for high-throughput functional characterization and discovery, both at the biochemical and cellular levels.

Synchrotron Technology Development

Development of new synchrotron technology, such as improving signal-to-noise and reducing radiation damage, can enable detection of low-occupancy ligands, a common problem during optimization of small molecule leads.

Workflows Integrating Experiments and Modeling

Currently, there are no fully integrated standardized workflows and processes for systems-level characterization and modeling of host-pathogen interactions. In addition, there is a need to increase interdisciplinary capabilities that involve close interaction between experimental scientists and modelers.

Modeling Across Time and Scales

Improved mathematical and simulation methods are needed to analyze and integrate ever-increasing amounts of data with varied structure to characterize pathogen function in the context of host interactions. One example is building time courses from cross-sectional infection data, such as single-cell assays. Also needed are methods to synergize and integrate systems biology approaches with mechanistic modeling for greater insight into pathogen biology. Connecting pathogen biology with existing models and data, including protein-phenotype information, gene-disease networks, and knowledgebases, will greatly accelerate the development of methods to determine mechanisms of pathogenesis. Additionally, multiscale pathogen models that link the molecular and population levels in epidemiological models would provide information about how potential variants might behave at a population level.

Global Understanding of Molecular Mechanisms Across Pathogens

Obtaining a more global picture of molecular mechanisms across pathogens would facilitate applying our knowledge to novel pathogens of concern, as would a deeper understanding of the range of possible viral life cycles and evolutionary relationships across hosts as diverse as bacteria, plants, and fungi.

Molecular Therapeutics Design and Prediction

Molecular therapeutics design is a multiparameter optimization problem in which the designed candidate must satisfy numerous objectives, including efficacy, adsorption, distribution, metabolism, excretion, safety, and manufacturability. Pharmaceutical companies traditionally optimize these objectives serially in a process that often takes 15 years. Many groups are adding AI/ML algorithms trained from these multiple objectives into the design process to parallelize optimization of compound properties to truly shorten the design-to-market timeline.

Ultimately, the use of prediction (i.e., physics-based, ML, and AI approaches) and high-throughput nanoscale technologies for each aspect of drug design and development can accelerate the drug design process. Continued support in building physics-based, ML, and AI models is critical, but the lack of data needed to create biologically relevant computer models hinders predictions. New instrumentation and experimental protocols in high-throughput nanoscale assays could deliver the necessary data to enhance prediction capabilities and provide validated results to accelerate the process. However, a true paradigm shift is needed to overhaul the overall drug design and development process to shorten the time to market during a pandemic. Needed advancements include creating parallel workflows and forcing late-stage druggability requirements into early stage design.

Target Identification and Broad-Spectrum Therapeutics

Improvements in target identification are still needed, especially if a broad-spectrum approach is desired. Molecular mechanisms and systems biology approaches are necessary for identifying critical protein targets that represent nonredundant pathways, as typical in biological systems. Once identified, typical molecular therapies start with a screening process, whether experimental only or experimental with computational methods. During COVID-19, virtual screening and computational optimization of hits to leads improved significantly, but validated assays for experimental screening were often a limitation. As part of the SARS-CoV-2 effort, small-molecule experimental screening campaigns were undertaken across multiple facilities using a wide range of approaches, many based on commercially made assay kits and reagents. For instance, numerous protease assays were readily available, including multiple substrate peptides with and without labels, known proteolytic inhibitors, and control proteolytic enzymes. However, these assays were validated to other well-studied proteases and needed to be tested and optimized for use in assessing SARS-CoV-2 proteases and related host membrane-bound receptor interactions. Many such

commercial assays were not readily available for specific or novel proteins identified from SARS-CoV-2. Similar issues exist for cell-based assay development, which requires identifying the best cell type and cell response readout. These activities mean that extensive amounts of time and potentially increased costs are required to first understand the assays' utility and robustness (sensitivity and specificity) as well as false-positive and false-negative rates.

Availability of Samples and Materials

The COVID-19 pandemic amplified many of the historical bottlenecks in drug discovery and development, such as synthesis of compounds or biologics, especially novel compounds. Specifically, material acquisition or creation was delayed, and chemical shortages limited production. Other challenges in characterizing factors important for compound druggability, such as toxicity, tissue specificity, and cell-membrane permeability, delay successful therapeutic development. Technological advancements are needed in all these areas.

Science to Predict Human Outcomes

Development of leads to druggable compounds is the true bottleneck of any molecular therapy program. Hundreds of millions of dollars are spent making drugs safe for patients (Watkins 2011). With failure rates of >70% in clinical trial 1 (safety), the experimental models used during development are only 30% predictive of human outcomes. These models include cellular and organoid/organ-on-a-chip assays and animal models. Improvements are needed in this entire area of science to make accurate predictions of human outcomes. Additionally, adding predictive power in the design phase of compounds for human outcomes would improve the success rate of compounds getting through clinical trials.

D4. Epidemiological and Event Modeling for Response and Recovery

As the COVID-19 pandemic unfolded, the epidemiological modeling community mounted an impressive response to support decision-makers with forecasts of disease progression. Forecasts at the national, state, and county levels have been common, with fewer forecasts being made at the city or metropolitan level. The U.S. government recognizes that accurate and interpretable event and epidemiological modeling is critical for effective decision support (USGAO 2020), and this recognition has been confirmed during the COVID-19 pandemic. Epidemic and event modeling has also been highlighted as a priority area in both the Biden administration's recently released pandemic preparedness plan, "American Pandemic Preparedness: Transforming Our Capabilities" (White House 2021) and in the National Science and Technology Council's newly published "Plan to Advance Data Innovation" for epidemic modeling and forecasting (White House 2022).

Epidemiological Modeling—Background

Mathematical and statistical models for infectious disease have been instrumental in providing critical understanding, informing mitigation, and eradicating diseases. Epidemiology has progressed dramatically, beginning with John Snow's analysis identifying a contaminated well as the source of London's cholera outbreak in the 1850s. The field advanced to make the discovery that malaria is spread by mosquitoes, and Ronald Ross's early 1900 mathematical model showing the mosquito control level required to eliminate malaria remains foundational for nearly all disease transmission models today. Continued advancements in epidemiology eventually led to the global elimination of smallpox and rinderpest. Indeed, epidemiological modeling has been essential in providing decision-makers with required information, from determining the necessary level of vaccine coverage to stop measles transmission to quantifying the impact of genetically modified mosquitoes on dengue transmission.

The basic idea behind infectious disease models is that pathogen transmission, whether between plants, animals, or humans, can be determined by quantifying the following factors: (1) the way transmission occurs, (2) the rate at which transmission occurs, (3) the progression of an infection (often using the Susceptible-Infectious-Recovered/Immune [SIR] model or the Susceptible-Exposed-Infectious-Recovered/Immune [SEIR] model), and (4) the relevant host and/or vector dynamics through space and time. Pathogens and parasites are varied and highly adapted

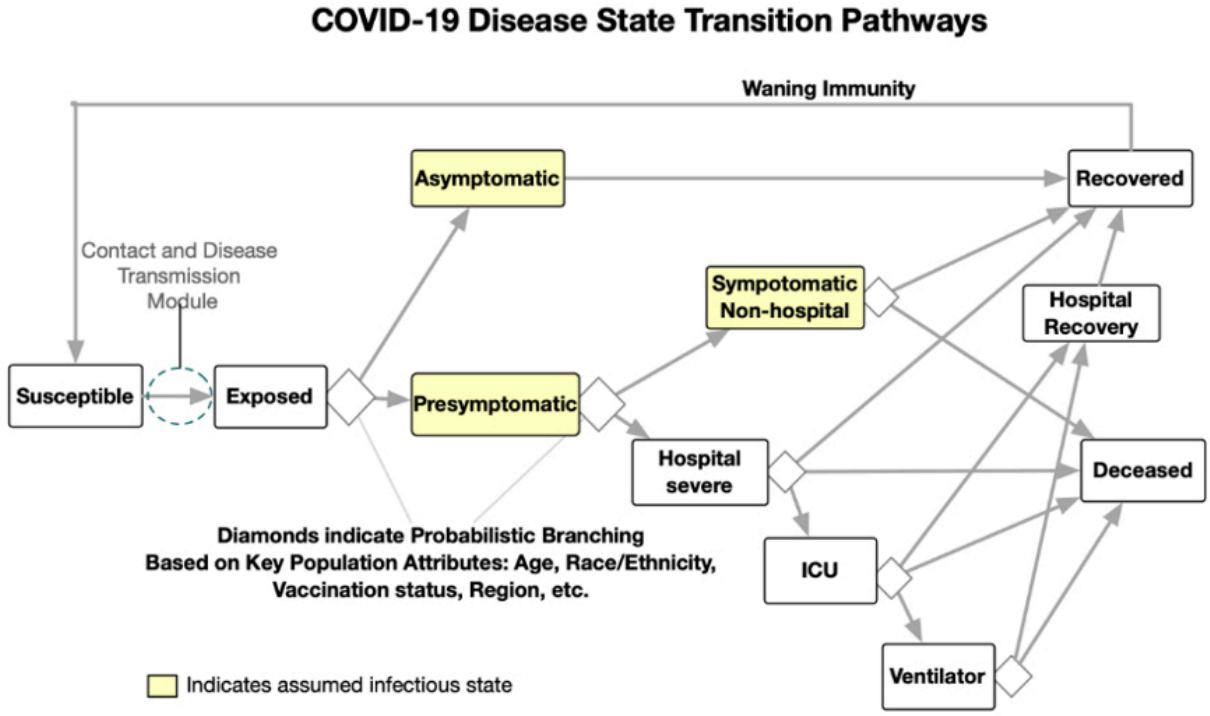

Fig. D6. COVID-19 disease states and transmission pathways.
[Image credit: Argonne National Laboratory]

for transmission through a range of mechanisms, including airborne (e.g., COVID-19 and flu), sexually transmitted, waterborne, vectorborne, foodborne, windborne, and other mechanisms. The spread of diseases through this broad range of mechanisms can be impacted not only by human behavior and infrastructure, but also by weather, climate, ecology, culture, as well as animal, plant, and insect biology and behavior.

Current epidemiological modeling of COVID-19 includes intrinsically different types of modeling approaches: (1) SIR/SEIR compartmental models based on differential equations that specify mathematical rates of change between population compartments; (2) agent-based models, in which the interactions of individual agents (e.g., people) and their explicit behaviors and contacts are directly simulated; and (3) statistical Bayesian forecasting or AI/ML models which make fewer explicit assumptions about the details of the disease and its transmission. The first two types of modeling approaches are often called "mechanistic" models because they explicitly represent and uncover the key variables in mechanisms of disease transmission. For example, Fig. D6, this page, shows detailed states and transition pathways for COVID-19, which can be implemented at the population level in compartmental models or at the individual level in agent-based models. Each of these approaches is capable of, for example, predicting the expected daily number of deaths, hospitalizations, and infections for COVID-19 in each geographical region, as well as providing uncertainty bounds for these estimates if stochastic elements are included in model formulations. No single model can answer all relevant questions, and, as in any modeling framework, every model has strengths and weaknesses in capturing the heterogeneous dynamics that determine important outcomes. Temporal factors such as time granularity dictate a model's ability to capture important dynamics affecting event timing, such as forecasting peaks and turning points. Models also vary in their ability to capture public response to health interventions and reported disease spread. Multiscale, multimodal modeling and data analytics are essential components of moving the epidemiological modeling field forward.

Epidemiological models and the healthcare systems they model are situated in a broader context of factors relating to human systems. These factors in turn have impacts on infrastructure, society, and the economy, including the previously often overlooked family units, childcare, school, medical, and other critical sectors necessary for basic needs. We also continue to see problems with supply chains, ranging from PPE, testing kits, and COVID-19 medications to basic food and consumer goods. Models are needed that can inform decision-making for health and infectious diseases from local and regional all the way up to national and global scales. Demand also exists for models to predict COVID-19 spread at the installation or building level and within individual rooms. The models need to be calibrated and validated with the best available data while acknowledging that the data is biased, spatially and temporally heterogeneous, and available at different scales at different times. This is a very challenging data fusion problem alone.

D4.1 Current Capabilities and State of the Art: Epidemiological and Event Modeling for Response and Recovery

The United States recognizes the broad need for pandemic preparedness and improving capabilities before the next pandemic or other biological threats emerge, upgrading our ability to respond to any possibility and to respond faster and better (White House 2021). A U.S. Government Accountability Office (GAO) report summarized the use of models within the Department of Health and Human Services (HHS), the Centers for Disease Control and Prevention (CDC), and the Office of the Assistant Secretary for Preparedness and Response (ASPR) during various recent epidemics (GAO 2020). Models have informed decision-making during and after outbreaks of Ebola, Zika, pandemic influenza, and others, including public health planning, outbreak response, and, to a limited extent, resource allocation. The value of combining forecasts from an ensemble of models that vary across methods, complexity, data used, etc. (as is done in ensemble weather forecasting) has been duly recognized. Modeling efforts for COVID-19 continue to support decision-makers as well as the public at global, national, and state levels. In many cases, these models are developed and supported by university or laboratory research centers, such as the well-known Institute for Health Metrics and Evaluation (IHME) model (IHME COVID-19 Forecasting Team 2020). The National Institutes of Health (NIH) sponsors a research network for epidemiological modeling that has a portion devoted to COVID-19 called the Modeling Infectious Disease Agent Study (MIDAS) program. The CDC maintains the COVID-19 Forecast Hub, which provides a collaborative, open-science framework that welcomes participation from modeling teams around the globe to submit predictions from their best COVID-19 models (Cramer et al. 2021). The Hub includes daily updated forecasts from 46 modeling groups.

Below is a summary of current capabilities and state of the art for epidemiological and event modeling, including examples of DOE laboratory capabilities, where applicable, in the following key capability areas:

- Disease transmission models at various granularities (individual to population scale), incorporating available real-time data streams on surveillance, mitigation, and intervention;
- Individual-scale immune systems and pathogen dynamics, modeling individual-level immune system and pathogen dynamics, and genetic evolution of pathogens, locally and globally, and possibly gain of function;
- Event modeling for decision support, capturing real-time data streams into models, and providing decision-makers to timely access to forecasts and the effects of proposed interventions;
- Human and host behavior and associated socio-demographics (e.g., poverty, underlying health conditions, essential workers, etc.), and risk factors impacting transmission at various granularities (e.g., mask wearing or individual protection, critical sectors, long- and short-distance travel, holidays, medical care seeking, vaccine acceptance);
- Impacts to animals and plants. Animals, plants, and the environment are part of the human disease ecosystem. In addition, some pathogens can impact animals, plants, and/or the environment,

causing significant economic, health (e.g., food availability), and social disruption.

- Cascading impacts of disease spread on critical infrastructure and supply chains. Decision-makers must understand the indirect and secondary consequences of policy options for pandemic disease mitigation; and
- Validation and uncertainty quantitation. Proper capture of scientific-based uncertainties in all data, variables, and structural relationships through uncertainty quantification algorithms is essential to assess impacts of mitigations and decisions made by public health officials as well as the public, by running "what-if" scenarios at scale (e.g., augmented AI).

Disease Transmission Models at Various Granularities

Disease transmission models capture pathogen spread at the population scale. These include SIR compartmental, agent-based, and statistical models. Each of these modeling approaches is capable of, for example, predicting the expected daily number of deaths, hospitalizations, and infections for COVID-19 in a geographical region, as well as providing uncertainty bounds for these estimates, if stochastic elements are included in model formulations.

Compartmental models can run at local, state, and regional levels and are often relatively computationally inexpensive, because efficient differential equation solvers are standard in most coding languages. However, because of the high degree population aggregation and its heterogeneous characteristics, compartmental models are less useful at the national level. Compartmental models can supply quick responses to a limited number of "what-if" questions relating to events and possible interventions. They may be expanded to live on a network or grid with an SIR-model based at each node and connections between the nodes and grid cells that capture travel and movement impacts. Compartmental models retain the relative computational efficiency of a local SIR model while also being able to incorporate a fair amount of spatial and temporal heterogeneity (e.g., EpiGrid at LANL, GERMS at PNNL, Popflow at ANL, etc.).

Agent-based models (ABM) simulate individual agents and behaviors in the population with relatively little aggregation in space and time and can capture different specific heterogeneities, providing high-fidelity projections. ABM require more data to properly parameterize because of their increased granularity in time, space, and population representation. Calibration, simulation, and validation take longer and require extensive computational resources. However, the added granularity allows for detailed implementation of interventions and modeling behavior diversity throughout heterogeneous segments of the population, as seen in the extensive capabilities of ABMs such as Argonne National Laboratory's (ANL) CityCOVID and Los Alamos National Laboratory (LANL) and Lawrence Livermore National Laboratory's (LLNL) EpiCast. At Brookhaven National Laboratory (BNL), an agent-based model called Fluent has been adapted to COVID-19 using National Energy Research Scientific Computing Center (NERSC) computational capabilities. Several universities have large agent-based models contracted for use by government agencies (supported initially by NIH's MIDAS program). Examples also include the ABM EpiModel with Transportation (LANL's EpiCast + LBNL's BEAM), a DOE-funded project aiming to better understand the relationship between travel patterns and disease spread. While U.S. government agent-based modeling capabilities are advanced, most are constrained by current parameterization to a particular geographic location or pathogen.

Statistical forecasting and/or machine learning models include classical statistical approaches, machine learning and artificial intelligence, and many hybrid approaches that include both mechanisms and data-driven methods. Statistical forecasting models that predict cases, hospitalizations, and deaths have been widely reported on and have been very useful in the COVID pandemic. We are using the word "forecast" here to mean something like weather forecasting models, that give predictions, with associated probabilities, of what will happen in the short-term based largely on data and some underlying understanding of the systems. See, for example, the CDC's Influenza or Influenza-Like Illness (ILI) and now COVID-19 forecasting challenges that provide median forecasts of cases over the next several weeks, along with 95% confidence

bounds or intervals. However, the CDC currently relies on volunteers at various universities and organizations to provide forecasts and does not control who provides forecasts or when and how they stop providing forecasts. A fair amount of forecasting capability exists outside the federal government. DOE and the U.S. government in general have modest but powerful capabilities in (1) COFFEE (COVID-19 Forecasts using Fast Evaluations and Estimation) and predecessors at LANL (part of the CDC flu forecasting and COVID forecasting challenges); (2) Extreme-scale Model Exploration with Swift (EMEWS) at ANL; (3) in-progress machine learning disease forecasting models at Pacific Northwest National Laboratory (PNNL); and (4) PRIME, an open-source software package for computing short-term (7-10 days) forecasts of COVID-19 morbidity at Sandia National Laboratories (SNL) using time-series data on COVID-19 case counts.

Individual-Scale Immune Systems and Pathogen Dynamics

These models capture the infection of cells within a host and the host's immune response, as well as the effects of therapeutics, such as drugs or vaccines. They often capture the potential heterogeneity between individuals in terms of the course of an infection and the individual's infectiousness. In the case of chronic infectious diseases such as HIV, the within-host models may also include genetic mutation and evolution of the pathogen *within* the host. These models are important for quantifying the impact of individual medical interventions, as well as for understanding the disease trajectory critical to population-level transmission models. Within the federal government, LANL, for example, has developed world-renowned expertise in mechanistic models of within-host dynamics of multiple infections (acute, such as influenza, Zika or SARS-CoV-2; and chronic diseases, such as HIV, hepatitis B and C). This expertise is transversal from classical mathematical models (e.g., ordinary differential equations [ODEs] and partial differential equations [PDEs]) to computer simulations, and across multiple fields of experimental science, including virology, immunology, and experimental design. LANL has also interfaced within-host modeling with epidemiological models, for example, in HIV and SARS-CoV-2, but these efforts are still incipient. In addition, systems immunology modeling efforts (e.g., immune signaling, gene networks, gene drives, etc.) are also being conducted at multiple DOE laboratories and at NIH.

Models for the genomics of pathogens, their evolution, and the emergence of variants are important for assessing the impact of interventions, determining efficacy of vaccines and previous immunity, and anticipating changes in transmission dynamics. Selection pressure means that pathogens evolve to increase their fitness, including jumping to new species, evading immunity and vaccines, resistance to treatment (antimicrobial resistance), increased transmission rates, and more. Drug resistance in tuberculosis, malaria, and staph (MRSA), for example, is of major concern along with increasing antimicrobial drug resistance by many other pathogens. We have already seen SARS-CoV-2 evolve to become much more transmissible and to partially evade immunity from vaccines or previous exposure. Modeling gain-of-function as pathogens mutate is also needed to understand threats posed by possible mutations. These models are important for understanding and forecasting pathogen spread and severity, predicting efficacy of therapeutics, and developing vaccines. DOE has several world experts in this area. For example, LANL and ANL have demonstrated capacity in modeling infection flow between geographic units based on mutation patterns in pathogens sequenced from those regions. They also can establish phylogenetic clusters of closely related sequences and infer epidemiological relationships of individuals in those clusters (e.g., who infected whom and when), assuming pathogen sequences contain sufficient information. LANL also has pipelines for processing sequences at the millions scale to look for the presence of variants of concern or the presence of hidden outbreaks and to estimate certain parameters of interest (e.g., increased contagiousness) in those data streams.

Event Modeling for Decision Support

Across the U.S. government, models for a wide range of pathogens and systems including crops, livestock, wildlife, and human disease transmission have been used for decision support and basic research. Many of these are "one-off" models funded to consider a particular outbreak or concern as it occurs in real time. Examples include: (1) PNNL's Outside the

Continental United States International Travel and Contagion Impact Quick Look Tool (OIT-CI) used to study a cholera epidemic, (2) SNL's Facility Disease Control (FDC) model built in collaboration with the Office of Public Health of the Department of Veterans Affairs (VA) to optimize control measures for rapidly spreading diseases, and (3) LANL's EpiGrid and predecessors. A key question is: How can we build on these capabilities and be more agile in accurately responding to new outbreaks or pathogens? A valuable resource to address these questions would be a readily available and accessible library of models and parameters for a wide range of pathogen systems that could be pulled off the shelf as needed.

Medical and public health interventions and the public's reaction to them is an important modeling challenge, in addition to providing basic forecasts. While the models listed in the previous paragraph represent a basic capability, increasing their decision-making usefulness requires incorporating medical and public health interventions, including vaccines, medications, testing and surveillance, contact tracing, quarantine and isolation, mask wearing, social distancing, travel and movement restrictions, culling (in the case of crops and livestock), and more. Many of the models listed so far have the capability to include a subset of these possible interventions. However, addition of the many intervention options adds significant complexity and increases the number of parameters that must be fit or informed with data. As seen with COVID-19, interventions can also change rapidly across space and time, resulting in more challenging nonautonomous systems in terms of data and computational needs. We have some beginning capabilities to mine and assess data of local public health orders, including school closures, mask mandates, and business closures across DOE (and at Oak Ridge National Laboratory [ORNL], in particular). These kinds of centralized data of local decisions are both difficult to compile and very useful—if not necessary—for federal decision support. Related capabilities include models and tools at PNNL (International Travel to Community Impact [IT-CI]) and other decision-support tools; LANL (e.g., EpiCast, EpiGrid); ORNL models and tools; LLNL data assimilation capabilities; and other DOE laboratory capabilities.

Human Behavior and Socio-Demographic Modeling

Human behavior (or in some cases animal/insect/plant behavior) is another important area to include in models. Human behavior deserves its own section, particularly because, in addition to traditional data (surveys, studies, etc.), it requires the use of nontraditional data, including social internet, cell phone and location data, medical data (e.g., care-seeking, vaccines, therapeutics, outcomes), and more. Human movement, contact networks, and behavior vary across time and space and are quite complex and difficult to predict. ORNL has initiated work in quantifying how humans respond in real time (via Twitter or other social internet data) to public health orders, nonpharmaceutical interventions, and misinformation or disinformation. Current capabilities also include tools at PNNL, such as the Pandemic Discrete Event Simulation, which is a stochastic discrete-event simulation to model passenger screening for pandemic influenza at U.S. port-of-entry airports. Additionally, multiple projects at LANL use social internet data such as Twitter and Google Trends to quantify perceived risk, personal protection behavior such as mask wearing, and more.

Properly incorporating human socio-demographic factors and related human infrastructure (e.g., garbage collection, quality and density of housing, clean water, wastewater pathogens as indicators of disease spread, etc.) into epidemiological forecasting models is also important. Deciding which of these many factors are significant and which to use in a particular model for a particular pathogen is difficult and still largely an open question. Synthetic populations including detailed data on human populations are needed to inform understanding of how those demographics impact disease transmission and serious outcome risks (e.g., ORNL, UrbanPop and bundleUP). Co-evolution of social and ecological signals during a pandemic can also be included, and ORNL has developed initial research to explore these factors in models. NREL, LBNL, and ANL have incorporated at least minimal human demographics in multiple models previously listed.

Impacts to Animals and Plants

While most emphasis so far has been on human diseases, the critical impacts of animal (livestock, wildlife,

and companion animals) and plant (crop, wild) diseases on ecosystem health, food systems, and spillover to human diseases are underappreciated. Animals or the environment are often components of human disease systems (e.g., vector-borne disease spread by ticks and mosquitoes). The USDA has agricultural production systems models. For example, to inform decision-making related to horticultural crops, the Agricultural Research Service (ARS) has funded the development of models incorporating epidemiology, pathogen ecology, weather/climate, and more. In this area, LANL has been supporting the ARS-funded National Predictive Modeling Tool Initiative with analytics, databases, and forecasting tools. However, plant models are typically limited to economically important species, but noneconomically important species may have significant ecological value and other impacts. ARS also funds work to understand the production impacts of disease in economically important livestock species. For instance, ARS is investigating interactions and control measures for co-infections in catfish and building models to predict babesia (malaria-like protozoa) spread in the U.S., incorporating tick, wildlife, and livestock biology data, as well as climate and landscape data.

Greater awareness of and access to these modeling capabilities will be useful in preparing for and responding to future plant and animal disease events. SNL, PNNL, LANL, and others have existing models and expertise for zoonotic, wildlife, plant, and crop models at various scales and for a variety of pathogens. Examples include SNL's Dynamic Livestock Disease (DLD) model that generates quantitative disease spread predictions and addresses complex herd-mixing dynamics, migration, and transport, and LANL's EpiCast adapted to rinderpest. However, a critical need exists for a more complete set of model types for the full range of pathogen systems. The existing infrastructure for human disease modeling can lead the way in terms of what is needed for animal/plant transmission and critical infrastructure models.

Impacts to Critical Infrastructures

Decision-makers faced with highly complex alternatives for protecting our nation's critical infrastructures must understand the indirect and secondary consequences of policy options for pandemic disease mitigation before they enact solutions to prevent and mitigate disasters. The U.S. Department of Homeland Security (DHS) has identified a list of 16 Critical Infrastructure Sectors (CIS) where disruptions would have a debilitating impact on the nation's economy and impacts from events such as pandemics. Most critical infrastructures have interdependencies among themselves with many feedback loops. To address this need in the past, the Department of Homeland Security Science and Technology Directorate (DHS S&T) has developed tools to support decision-makers in reaching risk-informed decisions. With the addition of disease progression and epidemiology simulations and uncertainty analysis, decision support systems can have a unique ability to provide a high-level, integrated pandemic outbreak analysis that also represents critical infrastructure impacts.

Decision-makers can also be assisted in reaching the best coordinated responses by (1) the incorporation of national and metropolitan scale vantage points coupled with technical transparency and openness, (2) the treatment of uncertainty and sensitivity analyses, (3) the modularity of a system dynamics design, and (4) the ability to separate different consequences by turning on and turning off interdependencies. Examples of user-oriented features can include the model's ability to display information in units familiar to domain experts and to enable users to see the implications of assumptions on outcomes. For example, there are existing capabilities at PNNL, the SimWare Framework within which GERMS is integrated with models of critical infrastructure sectors and systems. A healthcare sector model not only allows for exploration of medical, pharmaceutical, and supply chain needs but also enables investigation of potential resource constraint impacts on treatment outcomes and the broader epidemic spread. Other capabilities include LANL's MEDIAN model with disease spread and infrastructure, SNL's Adaptive Recovery Model (ARM), and SNL's Medical Resource Demand (MRD) for COVID-19 treatment resource demands.

Validation and Uncertainty Quantification

All previously discussed disease transmission models perform better under certain circumstances and require various types and scales of data for calibration, validation, and uncertainty quantification (UQ). Uncertainty in disease transmission modeling can

largely be grouped into four categories: biological variation, policy differences, sociological response, and infrastructure response. During a pandemic, viral strains continue to evolve, altering contagiousness and virulence as a pathogen adapts. Biological variation is represented by the diversity of human populations in relation to immune function, health status, and gene function for infectious disease responses. Even as targeted vaccines are developed for a specific instance of a viral disease, the rapidity of viral evolution and the length of time required to develop and deploy a vaccine imply uncertainty in vaccine efficacy. The sociological response also creates extreme variability and uncertainties to a pandemic and resulting outcomes. In addition to investigating risks and mitigation strategies of vaccines, antivirals, and social distancing measures, assessing the potential range of pandemic consequences given the uncertainty about disease characteristics has become increasingly important. Simulation models combined with a rigorous computer experimental design with sensitivity analysis that incorporates uncertainty in the pathogen behavior and pandemic response can show the extreme variation that is possible. Investigating the range of outcomes that incorporates uncertainty in viral evolution, disease characteristics, mitigation possibilities, sociological responses, and public health policies can lead to a better understanding of overall probabilities for pandemic preparedness plan development. There is inherent knowledge that can be mined within the experienced variability.

It is critical to ensure that a broad range of models covering a broad range of pathogens stay up-to-date with people who know how to run them and can quickly adapt them. Equally as important as response is communication between government agencies in terms of what models are available to answer specific questions for specific systems. Of course, in epidemiological modeling, aleatoric (based on probabilistic variations) and epistemic (based on lack of knowledge about parameters and processes) uncertainties must be also acknowledged and addressed. Such uncertainties make it difficult to both predict what will happen under a particular decision and to attribute outcomes to a particular decision. More time should be devoted to exploring varying outcomes for identifying the most significant factors to make the best-informed decisions.

D4.2 Needs for Strengthening Biopreparedness: Epidemiological and Event Modeling for Response and Recovery

Epidemiological and Event Modeling in Decision-Making Support

Since the onset of the COVID-19 pandemic in March 2020, DOE laboratories successfully provided information to decision-makers at national, regional, state, and local levels. In so doing, the laboratories developed workflows that effectively incorporated available and emerging data and information on the SARS-CoV-2 virus and used a modeling process to translate that data into usable information for decision-makers. We identified workflow process gaps in terms of the methods, models, and supporting technologies that could make the process more timely, more efficient (using fewer resources), and more effective in the future. This section identifies those needs as a basis for discussion on improving future pandemic responses. Key challenges of interfacing with decision-makers are:

- Providing information on a timely basis, so it is still relevant in answering current questions and supporting decisions, and
- Providing information in a form that decision-makers can easily and efficiently understand, interpret, and use in the decision-making process.

Public health decision-makers ask a set of core questions during any outbreak, epidemic, or pandemic. For example, early in the COVID-19 pandemic, key questions were: (1) How many people will ultimately be infected, and when will the peak occur? (2) Are there interventions that will prevent the spread more effectively? and (3) How many people will require hospitalization and intensive care? Many of these questions were addressed by compartmental models having an aggregate level of detail on population and disease states. As the pandemic progressed, additional questions emerged, such as: (1) Given what is known and unknown about the virus and public response, what public health interventions might be taken? (2) Are

essential workers at excessive risk of being infected because of their public interactions? (3) What is the effect of social distancing practices? and (4) How quickly will the effects of vaccination be realized in reducing the spread?

Some of these questions may be highly granular in nature (e.g., agent-based), requiring fine-grained models to incorporate relevant variables for disease transmission. In this respect, such models are a continuing need. We must also be able to rapidly determine the granularity and type(s) of models required to answer the questions being posed. Finally, the ability to anticipate questions and needs based on up-to-date (near real-time) information combined with responsiveness to decision-makers is also essential. Access to a suite of models and experts would be helpful to determine the most important (and/or uncertain) parameters and to inform the experimental or field data necessary to reduce uncertainty and better inform models.

Needs to Strengthen Epidemiological and Event Modeling Capabilities

Improving epidemiological model interfaces to respond more quickly to decision-makers' questions include the following needs:

Real-World Modeling

To adequately respond to decision-makers, models must realistically represent aspects of pathogen spread through the relevant population(s) at a finer level of granularity and explicitly model the complex network of interactions that occurs between disease states, interactions among people, interventions, and geographical regions. Model detail or granularity is shown in three relevant dimensions: time, space, and population representation. Synthetic populations that better characterize whole populations are needed to understand their vulnerability to a disease and the factors impacting disease transmission as mediated by their place and activity. For example, early in the pandemic, important questions arose about vulnerable populations with pre-existing conditions (co-morbidity) making them especially vulnerable to contracting severe COVID-19. ML/AI algorithms operating on available, anonymized, and aggregate census data could be used to quickly develop synthetic populations whose characteristics adequately capture disease spread vulnerability with a high degree of reliability, obviating the need for data on actual individuals. In the future, information generated from modeling synthetic populations may include individual genome characteristics for understanding a population's susceptibility to new pathogens and variants. However, including within-host dynamics is a classical problem of multiscale modeling. As a result, the full power of within-host models has not been harnessed by epidemiological models. Nevertheless, it is possible that more realistic epidemic models could be developed with the inclusion of within-host dynamics. Multimodel approaches are also needed, including physics-informed ML/AI and similar approaches.

Adapting to the Real-World Situation

Real-world adaptation requires simple to use processes and systems for the timely reporting, collection, and integration of relevant data to enable effective modeling and forecasting for informed decisions. Thus, a challenge to epidemiological and event modeling is bringing real-world data into models in a timely fashion so that useful results are available to inform response and recovery. For example, as hospitalizations, deaths, and case reports are updated daily, models need to be recalibrated to reflect the most recent ground state. Models bring with them their own data requirements that may be outside the standard repertoire of available data, such as infectivity rates by age and other socio-demographic characteristics. ML/AI offer the possibility of inferring the most likely data values and uncertainty ranges for filling critical data gaps needed by epidemiological models. Quantifying uncertainty and bias in the data informing our models is also critical. Creating better partnerships between modelers, wet lab biologists, medical fields, and public health could also inform the data being collected and better account for data noise.

Conveying Information and Uncertainties to Decision-Makers and the Public

The form of information conveyed to decision-makers is critical to their acceptance of model results and continued long-term engagement with modelers. The information (e.g., charts, graphs, tables) modelers provide must be in a form that decision-makers are familiar seeing and interpreting. The information

should also be self-answering. In other words, it should answer anticipated questions of decision-makers, such as why the results came out as they did. An explanation that not only presents results but also explains them would be a good challenge for ML/AI to address.

High-performance computing (HPC) faces the challenge of propagating scientific-based uncertainties by running large numbers of scenarios in which assumptions about critical unknowns (e.g., behavior, population response to interventions) are varied across the full range of possibilities. For example, the portion of people who wear masks is a critical model parameter that cannot be predicted with certainty; however, model runs across the range of reasonable assumptions for mask wearing possibilities provide decision-makers with vital information that indicates the importance of mask wearing to limit the spread. Advances in emulators, HPC, and other new methods to expedite this process are needed.

Flexible Modeling to Meet Disease Requirements

The Australia Group's *Common Control List Handbook, Volume II: Biological Weapons-Related Common Control Lists* outlines several important pathogen and toxin-borne diseases that affect humans, animals, and plants and pose threats to society (Australia Group 2021). An epidemiological model could be developed for each biological threat that emphasizes the disease states relevant to the pathogen. Many variations of the basic SIR compartmental model are possible to reflect the relevant disease states for a specific pathogen. For example, for COVID-19, adding asymptomatic people is critical to understanding disease transmission by those showing no symptoms.

Many diseases are transmitted by disease vectors, such as insects, especially ticks and mosquitoes (e.g., malaria, Zika virus, West Nile virus, dengue, and yellow fever). Models that combine human and mosquito population life cycles (and how these cycles vary with environmental factors and climate) have been shown to effectively forecast vector-based disease transmission. As new diseases emerge and approach epidemic/pandemic proportions in the future, it is critical to have a flexible modeling workflow in which disease models can be quickly assembled and tailored to reflect the relevant disease states and transmission pathways.

Epidemiological Modeling in the Broader Environment

Within the healthcare system, epidemiological models interact with aspects of critical infrastructure, such as transportation systems and supply chains. Transportation systems are important venues for disease spread, depending on how the disease is transmitted. For example, airline travel and public transportation may offer transmission pathways for diseases spread through aerosols. In terms of supply chains, resources are required to operate the healthcare system as a system (e.g., critical care beds) and by the public (e.g., vaccines, masks, disinfectants). Epidemiological models, if properly structured, forecast the time-varying load of patients on the primary healthcare system and, implicitly, the required critical resources, such as PPE for healthcare providers, ventilators, etc. The implications of these resource requirements on supply chains provide crucial information for public health officials about potential shortages or delays in critical resource provisions.

Finding Better Interventions for Forecasting the Pandemic's End

Automated methods are needed to search the space of all possible intervention outcomes computed by epidemiological models and to identify those interventions most likely to reduce or curtail disease spread. Finally, modeling efforts related to disease events and surveillance are subject to the same boom/bust funding cycle that affects biodefense and public health emergency preparedness efforts. Ultimately, we need to combine genetic evolution, ecology, climate change, human impacts on and interactions with the environment, and other factors in pathogen emergence modeling to anticipate where and what kind of new pathogens are likely to emerge or re-emerge and cause regional or global pandemics.

Next Steps to Address Needs

Overcoming the needs identified above requires breakthroughs in the epidemiological and event modeling science and the use of the models in sustainable workflows, such as automating much of the process so it can be quickly performed and is both supportive

of and responsive to decision-maker needs. Given the combinatorically large number of interventions and possible combinations of interventions, along with the investigation of multiscale and multimodel approaches to understand real world situations, some research challenges in this area will require application of HPC resources.

D5. Materials and Manufacturing

The start of the COVID-19 pandemic was marked by urgent questions related to materials and manufacturing, including: How long can the SARS-CoV-2 virus remain infectious on surfaces? Are the materials in commonly available personal protective equipment (PPE) effective against transmission? How can manufacturing address acute shortages in masks, diagnostics, and ventilators with limited raw materials and workers? These questions exposed significant gaps in the fundamental understanding of pathogen-material interactions and pushed our capacity to rapidly develop innovative manufacturing processes and solutions. These uncertainties and needs also had significant effects on virus spread and public confidence in efforts to contain it. For example, widespread uncertainty about how long the virus is transmissible on surfaces undermined clear, evidence-based public health policy.

Meeting dire PPE and testing needs with hampered supply chains meant rethinking both technical processes as well as academic, government, and business partnerships. Such challenges required rapidly leveraging cutting-edge technical infrastructure, expertise, and production capacity across these different domains. Many of these questions and challenges have yet to be fully resolved, but as new biological threats emerge, they will need to be addressed. Preparing for future biological threats requires re-examining these and other questions related to materials and manufacturing and positioning our basic understanding, infrastructure, and workflows to answer key questions quickly and accurately about materials and manufacturing needs.

Here, we address the state of the art in understanding and shaping material properties that affect such interactions. We discuss their impact on the development and manufacture of critical technologies, including sensors and diagnostics, lab commodities, and PPE. Beyond material innovations, availability of these technologies depends on sufficient and innovative manufacturing capacity to enable adequate and precise production of technologies from such materials. This section discusses the impact of new process innovations and manufacturing strategies, such as 3D printing (i.e., additive manufacturing), that have been employed to meet demands. Looking beyond synthetic materials, we also consider the manufacturing infrastructure and innovative pipelines that have been utilized to meet growing needs for the scaled production of proteins, nucleic acids, and other bioactive compounds.

D5.1 Current Capabilities and State of the Art: Materials

How Pathogens Interact with Common Surfaces and PPE

The complexity of pathogen surfaces has limited theoretical frameworks for predicting pathogen binding to various materials. Pathogen-surface adhesion has typically been characterized empirically, with observations indicating that electrostatic interactions govern most adsorption events followed by van der Waals forces (Kimkes and Heinemann 2020; Zheng et al. 2021; Aydogdu et al. 2021). The exposed proteins and carbohydrates of pathogen surfaces vary widely, and their interaction with materials will also depend on degree of hydration. Viruses or bacteria in respiratory microdroplets, for example, will interact with surfaces differently than virions or spores as aerosols. The heterogeneity of pathogen surface chemistry has led to PPE effectiveness being determined on a case-by-case basis rather than being engineered for specific threats (Zhao et al. 2020; Hill et al. 2020; Zangmeister et al. 2020). Given the uncertainties of unknown pathogen-PPE interactions, novel mask designs based on general improvements in filtration media, manufacturing methods, innate antiviral properties, and reusability have been explored to combat future shortages (Wang, P. L., et al. 2021).

The molecular details of pathogen-material interfaces are critical to understanding the persistence and transmission of biological threats through the environment. While numerous methods exist for high-resolution

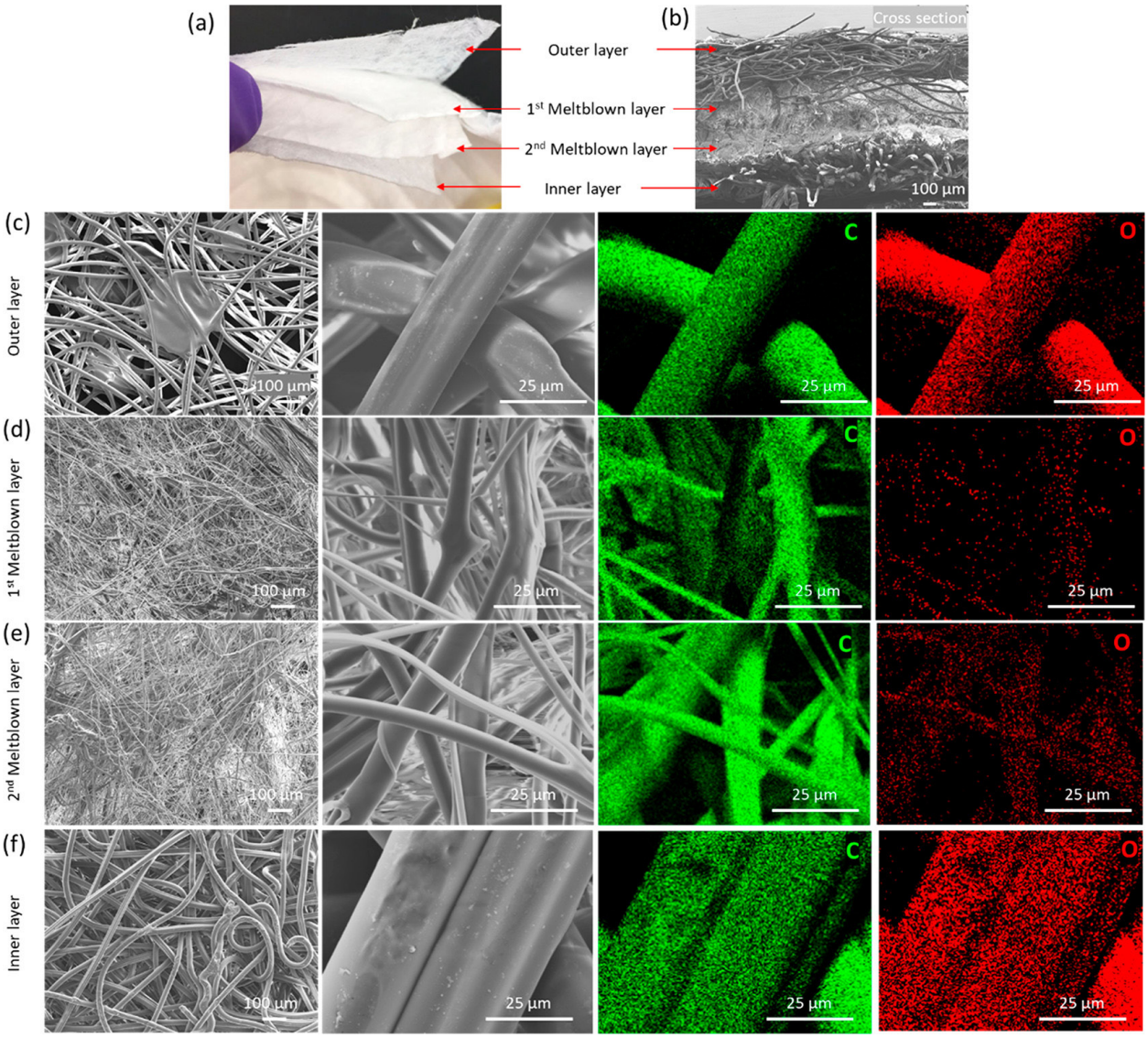

Fig. D7. High-resolution and atomic characterization of N95 masks. **(a)** Peeling apart 8210 N95 reveals four layers of nonwoven materials. **(b)** Scanning electron microscopy (SEM) cross-section image shows the outer layer, first and second middle melt-blown layer, and inner layer. SEM and energy dispersive X-ray spectroscopy (EDS) mapping images show top-down view of **(c)** the outer layer, **(d)** the first melt-blown layer, and **(e)** the second melt-blown layer. **(f)** SEM and EDS mapping of inner layer.

[Image credit: Reprinted under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) from Stackhouse, C. A., et al. 2021. "Characterization of Materials Used as Face Coverings for Respiratory Protection," *ACS Applied Materials Interfaces* **13**(40), 47996–48008. DOI:10.1021/acsami.1c11200.]

imaging of both materials surfaces and pathogens, capturing the molecular details of material-pathogen interactions has remained challenging. PPE surfaces like those in N95 respirators can be characterized by techniques such as scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS, see Fig. D7, this page), suggesting a basis of trapping virus particles smaller than 100 nm. Similarly, studies have characterized in detail the infection of mammalian cells with SARS-CoV-2 using high-resolution SEM (Caldas et al. 2020) and of free virus with cryo-electron microscopy and tomography (Ke et al. 2020). However, interactions between pathogens and surfaces present added challenges of imaging at

buried interfaces as well as working under ambient conditions needed to preserve pathogen structure. Ambient surface techniques such as atomic force microscopy (AFM) have been able to identify morphological differences between active and inactivated SARS-CoV-2 on mica but without details of virus-surface interactions (Lyonnais et al. 2021). Optical bioimaging methods, such as confocal microscopy, operate at resolutions (about 300 nm for visible wavelength) much larger than the size of a typical virus (<100 nm), and they generally require exogenous probes unable to access a tight interface. Multiphoton near-infrared excitation techniques are capable of subsurface imaging, including at deeply subwavelength resolutions (Lee et al. 2021), but probe access without disrupting the interface remains challenging.

Conventional optical imaging techniques, including fluorescence microscopy, can provide dynamic information about microbial and cellular attachment and viability on surfaces. However, mechanistic insights into the attachment and transformation of pathogenic viruses, and microbial pathogens to such surfaces, require tools that can capture material structure and biological detail at the molecular scale. Scanning probes and electron microscopies offer such detail, and with appropriate sample preparation techniques, they can be used to provide snapshots of protein, virus, and cellular attachment (Relucenti et al. 2021; Golding et al. 2016). Moreover, scanning probe measurements and AFM in liquid environments can extract mechanical information from living systems. Such measurements can be used to evaluate cellular responses to antifouling surfaces as well as antimicrobial and antiviral agents in real time (Hasim et al. 2018; Alsteens et al. 2013). Helium ion microscopes (HIMs) can offer some unique advantages over electron microscopes for characterizing the pathogen-material interface, including simplified sample preparation compared to that which is needed for electron microscopies to avoid charging. Ion milling (e.g., focused ion beam) can also be used in concert with imaging modes to alternate between imaging and material removal to provide three-dimensional tomographic information of the material interface in a manner similar to more mature dual electron/ion beam systems (Joens et al. 2013).

Materials in Integrated Sensing and Diagnostic Platforms

Materials play integral roles in any sensing and diagnostic platform—from their plastic housings to the microelectronics that control their operation to the sensing materials that exhibit unique optoelectronic properties when bound by a particular analyte. Describing these roles is beyond the scope of this document, but it is essential to consider biofouling and the stability of the biomaterial interface when developing any sensor or commodity component of a diagnostic system. The simple matter of making the analyte attach where it is supposed to turns out to be an incredibly challenging materials problem requiring control of surface topography and/or porosity, as well as chemical functionality and charge. Control of surface topography influences local hydrodynamic conditions and overall surface area, modulating the time that a given pathogen or target ligand is in close contact with the surface and overall surface area available for attachment. As described above, the interaction of surfaces with exposed pathogen proteins and carbohydrates is defined by electrostatic and van der Waals forces, as well as wetting of absorbent materials for pathogens in microdroplets. Surface energy (hydrophobicity and hydrophilicity) and charge modulate how surface pathogens with opposing or complementary surfaces adhere when they come into contact. Thus, selective modification of surfaces by targeting functional groups such as primary amines or carboxylic acids that decorate a fouling-resistant surface is a common route for functionalizing materials with complementary ligands used in sensing applications. For many sensing applications, polymers are ideal as raw materials or coatings that combine functional groups for selective modification while retaining a high density of hydrophilic groups (e.g., polyethylene oxide and polyethylene glycol) or other compositions known to resist nonspecific protein adsorption and nonspecific pathogen attachment (Maan et al. 2020). Similarly, significant advancements in sensor development have been achieved through self-assembled monolayers that readily enable the fouling-resistant chemical modification of pristine gold, silicon dioxide (and glass), and alumina, which can still be functionalized with select targeting ligands (Chaki and Vijayamohanan 2002).

Combined with nanofabrication techniques to pattern these materials or lithography techniques to pattern and modify self-assembled monolayers, new sensor arrays have been realized, along with fundamental insights into the attachment and persistence of cells, viruses, and proteins on surfaces (Whitesides et al. 2001; Schvartzman et al. 2011; Yu et al. 2013).

Materials for Air Filtration and PPE

The development of filtration media used in PPE, such as filtering facepiece respirators (FFRs) or building ventilation filters, depends primarily on fibrous textiles produced from synthetic and natural materials. The filtration efficiency—defined as the percentage of particles of a specified size and composition that are captured by the material under a specified pressure drop—depends on fiber diameter and arrangement and material thickness. Porosity, a parameter that describes the combined effects of fiber diameter and packing density, along with fiber arrangement and material thickness, can be altered to improve the filtration efficiency but comes with a trade-off in the form of an increase in pressure drop (i.e., breathability). Melt-blown polypropylene (PP) is the workhorse of the filter production industry, and its wide availability, good mechanical strength, low melt-temperature, low density, and hydrophobic surface energy make it ideal for low-cost filter production. Most importantly, the formation of electrets upon exposure to a corona discharge allows significant improvements in filter efficiency without commensurate increases in pressure drop, allowing for the creation of breathable, yet efficient filters. Efforts have been undertaken to improve efficiency by incorporating charge-enhancing additives in melt-blown isotactic PP (Larsen et al. 2021). Beyond the use of single-component polymers in fibrous filters, various levels of improvement in material performance and mechanical stability have been exhibited by electrospinning of composite filters from multiple types of polymer fibers, polymer fiber–organic particle blends, and hybrid polymer-inorganic particles (Huang et al. 2003). Electrospinning is a room-temperature process that can incorporate polymeric materials with high melting and glass transition temperatures.

As described above, most commercial N95 respirators use PP fiber-based filter media fabricated by melt-blown technology, in which the fiber diameter is 1-20 μm. These micrometer fiber networks alone cannot block fine particulates, and thus fibers are typically charged by corona discharge or triboelectric means to electrostatically trap small particulates. When disinfecting or sterilizing the electret FFRs for reuse, the treatment may damage the static charge of the fibers and cause filtration degradation. Thus, an appropriate treatment method should be selected. Various disinfection methods have been evaluated in this context, including steam, dry and humid heat, autoclave, alcohol and hydrogen peroxide, chlorine solution spray, and ultraviolet (UV) light (Ou 2020; Liao 2020; Baluja 2020; Carrillo 2020; Weaver 2020; Czubryt 2020; Cramer 2021; Jatta 2021). The results have varied considerably due to different testing conditions, such as chemical concentration, treatment temperature and humidity, treatment time, radiation intensity, and number of cycles. In general, dry heat at a temperature <100°C is safe regarding filtration properties and respirator structural integrity (Liao 2020). Humid heat at <85°C for a short time does not impact filtration properties as long as water condensation along fiber surfaces does not form (Liao 2020). However, humid heat treatment may cause failure of respirator fit (Ou 2020). Common liquid chemical soaking or sprays degrade filtration efficiency due to fiber depolarization (Liao 2020; Ou 2020). UV-C radiation has not typically been recommended because of a shallow UV penetration depth and the breaking of PP chemical bonds with accumulated radiation dose (Ou 2020; Liao 2020). To compensate for the charge loss during disinfection, recharging of the respirators after disinfection has been tested and showed positive results (Pirker 2021; Wang, P. L. et al. 2021).

Since performance deterioration of electrically charged filter materials is mainly due to charge dissipation, nonelectret filter media have also been developed using electrospinning technology (Zhang 2020; Leung and Sun 2020; Wang, P. L., et al. 2021). The electrospun fibers are typically less than 1 μm in diameter. Their network structure can facilitate Brownian motion of nanoparticulates within the filters, generating a strong diffusion effect and a high filtration

efficiency. Preliminary studies have demonstrated that electrospun media with a 95% filtration efficiency did not show efficiency degradation after 10 rounds of autoclave sterilization (Lograsso 2021). This indicates that nonelectret ultrafine-fiber filters can serve as good candidates for reusable FFRs.

In addition to reusability, electrospun media also have the advantage of reduced thickness (e.g., a few tens of micrometers) due to their high filtration efficiency. This favors easier respirator face fit and improved wearing comfort as compared to melt-blown filter media (which is often >200 μm thick). These features significantly expand the potential for using materials that can resist high-temperature virus inactivation by microwave, are compatible with the integration of antiviral and antibacterial ingredients (Wang, P. L. et al. 2021), and can even employ biodegradable materials such as polyvinyl alcohol and cellulose acetate.

Materials with Antiviral and Antimicrobial Properties

Pathogens can remain viable on surfaces long enough to permit transmission, and this timing depends on the material and its porosity, as well as on environmental factors like temperature and humidity (van Doremalen 2020; Chatterjee 2022). Because of this uncertainty in pathogen viability, use of medical protection tools, such as face masks and respirators, can lead to virus transmission from shared PPE or touch transfer. Adding antimicrobial and antiviral functions to PPE materials to inactivate or immobilize virus and bacteria can enhance protection by mitigating the risk of touch transfer and reducing the need for potentially performance-degrading sterilization techniques. Virucidal and bactericidal components can be synthetic nanostructures (e.g., metal nanoparticles, semiconductor nanoparticles, or carbon allotropes), simple salts, and photodynamic organic compounds (Zhou 2020; Wang, P. L. et al. 2021). Natural biodegradable materials, such as licorice root extracts, may reduce impact on the environment (Chowdhury 2021) and have shown virucidal potential (Cinatl 2003; Fukuchi 2016). Possible mechanisms for antiviral function may include linking and inhibiting virus attachment and penetration into cells, destroying the structure and function of viral proteins and nucleic acids through oxidation and radical reactions, increasing the immune response of the host cells, and inhibiting virus budding (Zhou 2020). These additives can be integrated into masks and FFRs during filter membrane synthesis (e.g., by introducing additives to the precursor materials of electrospun fibers (Wang, P. L., et al. 2021) or after membrane fabrication by surface coating via atomic layer deposition (Mane 2021), impregnation (Hewawaduge 2021), and spray coating technologies.

Materials for Pathogen Sensing

Wearable protective equipment capable of noninvasive monitoring of exposures is a potential avenue to curb transmission during biological events. Material strategies are currently limited for advanced or smart PPE (Shi et al. 2021) that offer both protection and produce useful and actionable diagnostic information. Materials development and engineering for wearable sensors involve detailed consideration of biosensors, devices, and the integrated garment. Wearable synthetic biology sensors have been demonstrated that include freeze-dried, cell-free genetic circuits integrated into textiles for biomolecule detection, while face masks with CRISPR-based sensors for detection of SARS-CoV-2 have been demonstrated to show good detection limits (Nguyen 2021). However, protection and sensing constructs may suffer in environmental conditions such as high humidity and typically are single use or have a limited operational time. Lab-on-a-glove concepts with integrated electrochemical sensors are viable for chemical detection, such as to target opioids, but offer few, if any, options for nucleic acid or protein targets. Future breakthrough designs for sensing or smart PPE are likely to be more robust yet have a low physical burden to the user, be faster in their detection time for early action, be well-integrated at the device and garment levels, and have low cost.

Opportunities to develop wearable technologies for continuous health monitoring have emerged through the exploration of stretchable and flexible hybrid materials and electronics composed of unique inorganic nanomaterials and functional polymers or glass-like matrices (Gao et al. 2019). Such innovations would dramatically shift approaches to healthcare management and human interactions during a pandemic. Early advances in this area were demonstrated with

carbon nanotube assemblies embedded in polymer matrices used to create transparent conducting electrodes; improved efficiency organic light-emitting diodes (OLED); artificial neural bundles; and sensors for monitoring changes in humidity, temperature, and pressure (Ivanov et al. 2012). In such applications, controlled dispersion and alignment of the nanomaterials within the matrix were critical to the emergence of unique thermal and electrically conducting properties. More recently, atomically thin 2D materials—such as graphene, hexagonal boronitride (hBN), transition metal dichalcogenides (TMDs), and their heterostructures—have offered an incredible array of optoelectronic properties and have been touted broadly for their potential impact on sensing and next-generation computing. These materials, combining unique tunable functionality, amazing strength-to-weight ratio, durability, and flexibility, are ideal for developing wearable multimodal sensors for comfortable, noninvasive, and continuous health monitoring and telemedicine (Zhang et al. 2021).

D5.2 Current Capabilities and State of the Art: Manufacturing

The rapid spread of COVID-19 resulted in significant supply chain issues with respect to critical medical supplies and medical equipment, especially PPE, diagnostic and test equipment and supplies, and clinical hardware. Shortfalls included N95 respirators and surgical masks, face shields, nasal swabs, and ventilators. Lack of a U.S. manufacturing industrial base in critical medical supply chains limited capacity to respond quickly and put medical professionals at risk, resulting in slower and less effective responses to the emerging crisis. State-of-the-art advances in additive manufacturing technologies—including extensive tooling infrastructure for 3D printing and prototyping, tools for logistics and supply chain management, and broader design capabilities—can be used to support manufacturing efforts at U.S. companies. Combined with a rethinking of public-private partnerships, these technical advances could translate into rapidly deployable solutions to meet dynamically changing needs during a biological threat event (U.S. DOE 2021; U.S. DOE 2022).

Rapid Prototyping

At the beginning of the COVID-19 pandemic, nationwide shortages surfaced in consumables used for testing. These shortages in critical components of viral testing kits included nasopharyngeal swabs, sample vials, and test tubes. Shortages resulted from the compounding effects of increased demand and decreased supply caused by the temporary shutdown of many production facilities due to sickness among large workforce populations. To bridge the gap between the demand and supply of nasopharyngeal swabs, the country quickly turned to temporary alternatives and new manufacturing methods and sources, such as 3D printing (Manoj et al. 2020; Tooker et al. 2021; LLNL 2022). For example, DOE national laboratories worked with a range of 3D printing companies and clinicians to design, print, and test replacement swabs. National laboratory facilities and staff provided services such as mechanical testing, sample-collection efficiency measurements, and biocompatibility assessments. Results were broadly provided to the community through timely release of reports and data. Similarly, test tubes and vials were in short supply, and the national laboratories provided rapid prototyping services for design iterations by industry partners.

Supporting and Augmenting Manufacturing Supply

Implementing a comprehensive approach to supply issues, beyond advances in tooling to simply scale output, is essential during a biological event. For example, state-of-the-art advances in ventilator supply-chain analysis tools were instrumental in expanding ventilator availability during the early stages of the COVID-19 pandemic. These tools analyzed the supply chain for existing ventilators, helped industry partners locate critical components, and supported market prediction and order fulfillment. Furthermore, techniques to alter and modify equipment built for other purposes can also increase capacity for uses specific to a biological event response. For example, the national laboratories created a pathogen management kit that could convert a Phillips Respironics V60 BiPAP to a full-scale ventilator, ultimately increasing ventilator supply.

Accelerating Translation Through Public-Private Partnerships

Advances in manufacturing tooling, process design, and supply chain management developed in government and academic settings rely on continuous feedback and strong collaboration with industrial partners that have the capacity to utilize and deploy solutions at scale. During a biological event, established public-private partnerships have been crucial in accelerating deployment of new materials and products for responding to the national crisis. For example, during the COVID-19 pandemic, a public-private partnership involving DOE national laboratories explored new high-throughput blow forming of test tubes at a Coca-Cola Company facility and used the technique to increase test tube availability. This successful collaboration exemplifies how unique partnerships combined with advanced process engineering can be used for rapid repurposing of existing facilities in times of need. Similar public-private partnerships involving DOE national laboratories contributed significantly to the COVID-19 response, including (1) enabling production of up to 10 million test kits per week, (2) expanding domestic production of N95 materials to support production of more than 3 million masks per day, (3) validating additively manufactured swabs allowing production of up to 250,000 swabs per day, and (4) developing and commercializing a new ventilator that received U.S. Food and Drug Administration (FDA) emergency use authorization (U.S. DOE 2021; U.S. DOE 2022).

Manufacturing of Biologics and Therapeutics

Following FDA recommendations, pharmaceutical companies can begin exchanging traditional batch chemistries for continuous-flow approaches that offer superior reliability and control. Instead of traditional scaling up, duplication of smaller reactor units (i.e., scaling out) has been adopted for achieving the same manufacturing scales, and scaling out can provide more precise control over reaction conditions. Additional state-of-the-art developments in reaction engineering include real-time process analytical technology (PAT) to ensure process quality control as inline analytic data for a given process. Inline analytics are particularly useful in providing information about product identity and purity. Technology supporting inline analytical capabilities continues to advance with greater portability, which is useful for plug-and-play to synthetic flow reactors. Recently, the enabling technologies of continuous-flow reactor systems and PAT have been deployed to assist with synthesis and characterization of small molecules for treating COVID-19. As part of a recent initiative funded by the Defense Advanced Research Projects Agency, hydroxychloroquine was synthesized using continuous-flow reactors (Yu et al. 2018). Gilead has also published a continuous flow–based approach for producing Remdesivir (Vieira 2020).

Assuring fast and widespread access to life-saving therapeutics is a core challenge in any pandemic response. However, post-development, biotherapeutics manufacturing that follows current good manufacturing practices (cGMP) [e.g., monoclonal antibodies] is a resource- and skill-intensive process sensitive to microheterogeneity, process variation, and batch-to-batch variation (Gaughan 2016; Sardella et al. 2021). The pace of process development for biologics manufacturing, particularly in a pandemic-type emergency, may be hampered by (1) long lead times for milligram-scale DNA synthesis due to a limited number of synthesis service providers; (2) significant infrastructure investments, (3) stringent growth and sterility requirements, (4) lengthy production times, (5) bespoke process optimization requirements for mammalian cell production of biologics, and (6) onerous downstream processing requirements associated with products of mammalian hosts (Nadar et al. 2021).

Advanced manufacturing technologies are emerging that may ensure a resilient, agile, and flexible capacity to produce high-quality biotherapeutics (NASEM 2021). Several process innovations have the potential to dramatically accelerate the pace of biologics manufacturing to support rapid response efforts. These advancements enable distributed and modular synthesis of components or products, new routes and high-yield biologics production from tractable nonmammalian or cell-free systems achieved via process intensification or advanced process control, and intensified downstream processing and product assurance (Nadar et al. 2021). DOE national laboratories played a significant role in catalyzing innovations in these areas during the pandemic though their core

competencies and user facilities in synthetic biology and biomanufacturing, bioprocess development, and additive manufacturing. Many established fermentation processes used for biofuels or other products are applicable to the biomanufacturing of proteins and nucleic acids needed for diagnostics, vaccines, and therapeutics. Early in the COVID-19 pandemic, facilities were repurposed for biotherapeutic and vaccine candidates, such as Swiftscale Biologics, which worked with Lawrence Berkeley National Laboratory's Advanced Biofuels and Bioproducts Process Development Unit; this repurposed facility was able to develop a cell-free process that achieved a 1,000-fold increase in production volume of neutralizing antibodies, allowing the company to advance to clinical trials. Other repurposed scale-up manufacturing has been successful with CRISPR-based technologies for rapid COVID tests and nutritional enzymes to treat COVID patients (LBNL 2022).

D5.3 Needs for Strengthening Biopreparedness: Materials and Manufacturing

As demonstrated in Sections D5.1 and D5.2, the applications of materials and manufacturing to biopreparedness are vast, spanning PPE and pathogen interactions to diagnostics and instrument development, and they are critical for effectively mitigating the impacts of biological events. Likewise, there are diverse needs that could be addressed to strengthen biopreparedness through advances in materials and manufacturing.

Characterization of Pathogen-Material Interfaces

In addition to existing analytical capabilities, the development of new analytical tools for rapid characterization of pathogen structure, particularly for characterization of the pathogen-material interface under ambient conditions, would provide critical information for designing materials for diagnostics and PPE. Biosafety containment at the appropriate containment level for the pathogen (often BSL-3) is needed to conduct these studies. The characterization studies may also help to identify essential pathogen traits needed for designing pseudoviruses or other nonpathogenic surrogates, reducing demand on BSL-3 facilities and enabling wider study of novel pathogens. Critical methods for examining bonds between pathogens and common materials may include label-free X-ray scattering methods as well as synchrotron infrared, force microscopy, Raman, or other spectroscopic techniques. Optical probes specific for new pathogens would enable tracking of pathogen spread in aerosols and droplets, as well as from surface to surface, using more commonly available optical imaging techniques. Also potentially relevant are new methods to flash-freeze pathogen-material samples for these techniques or cryo-electron microscopy, which would obviate the need for instrumentation compatible with BSL-3. These techniques often have preferred substrates or grids, and efforts to develop instrumented nanofabricated platforms that enable *in situ* and *operando* studies in electron microscopes (EM) may be a path to enabling real-time visualization of pathogen-material interactions. Platforms using on-chip heating, fluidics, and electrical connections, along with ultrathin electron transparent windows, may allow EM visualization of pathogens within aqueous environments.

Modeling Pathogens on Surfaces

While there is a great deal of work on protein interactions with ligands and other proteins, there is little understanding of how pathogen surface proteins interact with synthetic materials. Theoretical frameworks and computational approaches need to be developed to understand the forces and bonds that govern interactions between pathogen surfaces and material surfaces. These interactions might include representative viral glycoproteins or bacterial receptors in conjunction with PPE materials or virucidal and bactericidal materials. Intensive calculations may leverage high performance computing capabilities to inform the design of materials to more effectively trap or inactivate pathogens.

Novel PPE Materials and Benchmarks for Performance

While some traditional PPE are effective in the current pandemic, novel pathogens may present added challenges for designing and manufacturing protective gear for widespread use. A central component of biomaterials preparedness is the exploration of innovative filtration media and respirator designs in anticipation

of a pathogen whose physical properties differ from well-studied organisms. For all PPE, there is a pressing need to develop a clear understanding of how these materials perform over repeated use or disinfection. Insights are needed about their response to changing environments, such as shifts in temperature and humidity, exposure to sunlight, and repeated mechanical handling. Beyond the mask materials that provide a basis for particle separation, components other than filters (including straps, nose pieces, and structural supports) also need to survive disinfection treatment and maintain good mechanical integrity. Commercial attempts have been made to fabricate reusable detachable N95 mask shells. The technoeconomic analysis of reusable FFRs should evaluate the economic feasibility of both reusable filters and reusable mask shells, as well as disinfection costs. Finally, regulatory guidance and operational protocols should be developed for disinfecting reusable FFRs using different decontamination methods to ensure safe and standardized practice.

Numerous research groups have tested various virucidal additives against different species, but variations in testing protocols make quantitative comparisons difficult, leading to potential discrepancies in inactivation kinetics for antiviral ingredients and integration processes. Benchmark testing protocols that define appropriate baseline measurements for efficacy would allow more meaningful comparison across different pathogens and materials. The structural integrity (e.g., nanoparticle binding to masks) and shelf life of antiviral masks and FFRs fabricated by different technologies have also been evaluated and noted as potential measures of value for material coatings and additives. Regulatory standards and guidance have yet to be provided.

Multifunctional Materials Design and Chemistries for Sensing

Widely available, rapid, and low-cost sensors for formats such as wearables or simple devices for home use require the integrated design of materials and chemistries. Features needed include specific binding of pathogens for detection while integrating transduction mechanisms and rapid chemistries to enable sensitive detection in one device. Examples include multifunctional materials that measurably change properties (e.g., optical or electrical) upon analyte binding to minimize the processing steps needed for simple and rapid detection. Multifunctional nanomaterials can provide options for integrating sample preparation (magnetic or solid phase separation) and detection via, for example, colorimetric, photoluminescent, electrochemical, and plasmon resonance methods (Vikesland and Wigginton 2010). However, research needs include increasing material stability for long-term use, combining nanomaterials with improved molecular recognition elements, using lower-cost materials with development for other markets, and creating new approaches for integrating sample preparation and detection to enable rapid, low-cost, and sensitive multiplexed detection.

Operando *Manufacturing Diagnostics*

Scalable manufacturing and integration of nanomaterials remain challenges and cost barriers to widespread delivery of advanced hybrid materials that contain functional nanomaterials (e.g., nanoparticles used for biocidal coatings and 2D materials for flexible electronic wearables). Investments in scalable and autonomous synthesis of these materials would enhance commercial viability while also aiding the discovery of new material combinations (i.e., 2D material heterostructures). Synthesis and manufacturing strategies that harness *in situ* and *operando* diagnostics to monitor material properties and adjust manufacturing parameters on the fly would improve product quality and reduce retooling and losses from faulty products. Moreover, an understanding is needed of how such materials are altered during large-scale production and integration processes. Scalable and environmentally friendly routes for synthesis, such as microbial synthesis of nanoparticles, may provide pathways for green manufacturing. New opportunities are potentially on the horizon with recent advances in large single-crystal synthesis of foundational 2D materials (including graphene and hexagonal boron nitride), combined with commercial endeavors to use large-scale graphene in real-world applications.

Low-Cost Globally Distributed Biomanufacturing

For pharma-grade products, cGMP requirements limit global distribution of low-cost vaccines and

therapeutics, potentially impeding pandemic containment. Nontraditional vaccine approaches, such as using probiotic bacteria engineered to express pathogen antigens to generate immune response, obviate the need for costly cGMP manufacturing capabilities. Engineered *Bacillus subtilis* strains have begun to show promise as oral vaccine candidates (Lv et al. 2020). For novel viral threats, prokaryotic expression of surface antigens, typically expressed by human host cells as membrane glycoproteins, remains challenging and needs a better scientific foundation for these approaches to be relevant. Solutions to these challenges may facilitate the critical pandemic response of rapid global vaccine and therapeutics deployment, including in countries that have minimal fermentation manufacturing capabilities.

D6. Summary

This report summarizes many of the current capabilities and state of the art in four key areas of pandemic preparedness: (1) surveillance, detection, and diagnostics; (2) molecular mechanisms, systems biology, and therapeutic development; (3) epidemiological and event modeling for response and recovery; and (4) materials and manufacturing. Extensive capabilities exist across all four areas, but much work remains to be done for robust pandemic preparedness to address the broad range of potential biological threats. This section outlines two central themes regarding needs for strengthening biopreparedness and highlights potential opportunities for DOE involvement.

One common theme that emerged was the need for technologies and solutions that can be rapidly tailored to address new and emerging biological threats. For example, threat agnostic approaches are needed for surveillance, detection, and diagnostics to enable early warning and effective detection and diagnostics for the wide range of known and emerging biological threats. Research is also required to enable the rapid design and prediction of effective therapeutics, along with the development of broad-spectrum therapeutics. Furthermore, advances in epidemiological and event modeling are essential for enabling model adaptation to real world situations and incorporating modeling of the broader environment. Characterization and modeling of pathogen-material interfaces, in combination with materials design and low-cost distributed manufacturing, are also necessary to ensure rapid design and production of materials that can meet critical needs, such as PPE, detection/diagnostics, and other biopreparedness requirements.

Another recurring theme was that collaboration, coordination, and communication are critical for effective biopreparedness. A convergence of multidisciplinary science, engineering, and innovation is critical to address biodefense research and development challenges. Effective biopreparedness also requires partnerships across federal departments, academia, and industry to generate a pipeline of solutions that span foundational research and innovation to development, applications, and operations. In addition, both experimental and computational capabilities must work together to advance biopreparedness—modeling should inform experimental research and experimental research should inform modeling. Furthermore, continued advancements in data analysis, AI/ML, and HPC will help accelerate the research, design, and prediction needed to address emerging biological threats.

DOE's NVBL research efforts and DOE's contributions to the COVID-19 HPC Consortium demonstrated how DOE infrastructure and capabilities contribute to all four pandemic preparedness focus areas discussed in this report. DOE experimental and computational user facilities and other capabilities could be further harnessed to help advance biodefense research areas essential for addressing and mitigating future biological threats. Future investments in infrastructure and foundational research for pandemic preparedness would also provide capabilities to not only meet national needs for a wide range of biological events impacting humans, animals, plants, and the environment, but also advance preparedness to address other crises impacting health, the economy, and security.

Appendix E

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Appendix F

Acronyms and Abbreviations

1D, 2D, 3D, 4D	one-, two-, three-, four-dimensional
3CLpro	main protease
ABM	agent-based model
ACE2	angiotensin-converting enzyme 2
ADME	absorption, distribution, metabolism, and excretion
AFM	atomic force microscopy
AI	artificial intelligence
ALS	Advanced Light Source
AMS	accelerator mass spectrometry
ANL	Argonne National Laboratory
APS	Advanced Photon Source
ARM	Adaptive Recovery Model
ARS	Agricultural Research Service, USDA
ASCR	DOE Advanced Scientific Computing Research program
ASPR	Office of the Assistant Secretary for Preparedness and Response
BER	DOE Biological and Environmental Research program
BES	DOE Basic Energy Sciences program
BNL	Brookhaven National Laboratory
BSL	biosafety level
CAMS	Center for Accelerated Mass Spectrometry
CASP	Critical Assessment of protein Structure Prediction
CDC	Centers for Disease Control and Prevention
CFN	Center for Functional Nanomaterials
cGMP	current good manufacturing practices
CINT	Center for Integrated Nanotechnologies
CIS	Critical Infrastructure Sectors
COFFEE	COVID-19 Forecasts using Fast Evaluations and Estimation
COVID-19	Coronavirus disease 2019
CNM	Center for Nanoscale Materials
CRISPR	clustered regularly interspaced short palindromic repeats
cryo-EM	cryo-electron micro scopy
DARPA	Defense Advanced Research Projects Agency
DHS	U.S. Department of Homeland Security
DHS S&T	DHS Science and Technology Directorate
DLD	Dynamic Livestock Disease
DLS	Diamond Light Source
DoD	U.S. Department of Defense
DOE	U.S Department of Energy
EDGE	Empowering the Development of Genomics Expertise
EDS	energy dispersive X-ray spectroscopy
EM	electromagnetic
EMEWS	Extreme-scale Model Exploration with Swift
FDA	U.S. Food and Drug Administration
FDC	Facility Disease Control
FEMA	Federal Emergency Management Agency
FEVER	Fast Evaluation of Viral Emerging Risks

FFR	filtering facepiece respirator
GPS	global positioning system
hBN	hexagonal boronitride
hERG	human ether-a-go-go-related gene
HFIR	High Flux Isotope Reactor
HHS	U.S. Department of Health and Human Services
HIM	helium ion microscope
HPC	high-performance computing
iPSC	induced pluripotent stem cells
IHME	Institute for Health Metrics and Evaluation
IR	infrared
IT-CI	International Travel to Community Impact
IUPAC	International Union of Pure and Applied Chemistry
LAMP	loop-mediated isothermal amplification
LANL	Los Alamos National Laboratory
LBNL	Lawrence Berkeley National Laboratory
LCLS	Linac Coherent Light Source
LDRD	Laboratory Directed Research and Development
LIBS	laser induced breakdown spectroscopy
LLMDA	Lawrence Livermore Microbial Detection Array
LLNL	Lawrence Livermore National Laboratory
MD	molecular dynamics
MEDIAN	Modeling Epidemics for Decision Support with Infrastructure Analysis
MIDAS	Modeling Infectious Disease Agent Study
ML	machine learning
MRD	Medical Resource Demand
mRNA	messenger RNA
MRSA	Methicillin-resistant *Staphylococcus aureus*
NCBI	National Center for Biotechnology Information
NERSC	National Energy Research Scientific Computing Center
NIH	National Institutes of Health
NLM	National Library of Medicine
NNSA	National Nuclear Security Administration
NREL	National Renewable Energy Laboratory
NSLS-II	National Synchrotron Light Source-II
NVBL	National Virtual Biotechnology Laboratory
ODE	ordinary differential equation
OIT-CI	Outside the Continental United States International Travel and Contagion Impact
OLED	organic light-emitting diodes
ORNL	Oak Ridge National Laboratory
OSTP	Office of Science and Technology Policy
PanDDA	Pan-Dataset Density Analysis
PAT	process analytical technology
PDE	partial differential equation
PLpro	papain-like protease
PNNL	Pacific Northwest National Laboratory
PP	polypropylene
PPE	personal protective equipment
QUEST	Quantification of Uncertainty in Extreme Scale Computations
QC	quality control
qPCR	quantitative polymerase chain reaction
R&D	research and development
RBD	receptor-binding domain

RNA	ribonucleic acid
RPA	recombinase polymerase amplification
RTC	replication-transcription complex
RT-LAMP	reverse transcription loop-mediated isothermal amplification
RT-PCR	reverse transcription polymerase chain reaction
RT-qPCR	reverse transcription quantitative polymerase chain reaction
SANS	small-angle neutron scattering
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SAS	small-angle scattering
SAX	small-angle X-ray scattering
SC	DOE Office of Science
SciDAC	DOE Scientific Discovery through Advanced Computing program
SEM	scanning electron microscopy
SIR	Susceptible-Infections-Recovered
SEIR	Susceptible-Exposed-Infectious-Recovered/Immune
SNL	Sandia National Laboratories
SNS	Spallation Neutron Source
SPR	surface plasmon resonance spectroscopy
SSBD	structure-based drug design
SSRL	Stanford Synchrotron Radiation Light Source
STEM-EDS	scanning transmission electron microscopy–energy-dispersive X-ray spectroscopy
TMD	transition metal dichalcogenide
TMF	The Molecular Foundry
uHTVS	ultra-high-throughput virtual screening
USDA	U.S. Department of Agriculture
USGAO	U.S. Government Accountability Office
UQ	uncertainty quantification
UV	ultraviolet
VA	U.S. Department of Veterans Affairs
VECMAtk	Verified Exascale Computing for Multiscale Applications toolkit
WHO	World Health Organization

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