Perspective

Chemical Engineering and Virology: Challenges and Opportunities at the Interface

John Yin
Dept. of Chemical and Biological Engineering, 1415 Engineering Drive, University of Wisconsin-Madison, Madison, WI 53706

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Introduction

We live in an interesting time where chemical engineering research and applications are focusing ever-greater efforts toward the design, synthesis and characterization of materials at the nanometer scale. At the same time, cellular and molecular biology, which have historically worked to elucidate how the molecules of the cell work, increasingly seek to explain how interactions among these molecular parts define integrated processes that are essential for life. As chemical engineering and biology converge, engineers and scientists from both fields are enriched and inspired. Here we highlight how virology—the study of viruses—is opening new challenges and opportunities in chemical engineering research.

Viruses are naturally occurring nanometer-scale particles made by living cells. They cause a variety of human diseases including acquired immunodeficiency syndrome (AIDS), influenza, hepatitis, cancer and the common cold. The majority of viruses fall in a size range from 10 to 200 nanometers in diameter, where each virus particle carries its own genome made of either DNA or RNA. These genomes are protected from the environment by an outer shell composed of multiple copies of the same or similar proteins.

Are viruses alive? The answer depends on how one defines "alive." Viruses certainly share features with established life forms: they reproduce, they carry genomes that encode essential functions for their growth, and their populations evolve to changing environmental conditions. However, unlike microbial cells all viruses need externally provided pools of raw materials, such as amino-acid monomers to make proteins and nucleic-acid monomers to make DNA or RNA. Bacteria can synthesize these components from simple carbon and nitrogen sources, such as glucose and ammonia, but viruses cannot. Moreover, all viruses need to make proteins, but none carry or encode the complex machinery that bacteria and higher organisms make and use for manufacturing proteins. Thus, viruses grow and persist by a "hand-to-mouth" strategy that relies on pirating the material and energy resources of their living cellular hosts (Figure 1).

J. Yin's e-mail address is yin@engr.wisc.edu.

Viruses as engineered products

Despite their bad reputation for causing disease, viruses can be beneficial for human or animal health. Some vaccines, such as those that stimulate our immune defenses against infection by polio or measles viruses, are based on the manufacture of weakened or inactivated virus. In addition to vaccines, emerging virus-based products of the future will include viruses that have been genetically altered for uses in gene therapy. Here the motivation is to develop therapeutic approaches that address genetic defects in living cells or tissues. The strategy is to deliver to these defective cells or tissues genetic material (DNA or RNA) that supplements missing essential functions or shuts down defective ones. Examples include applications in the treatment of cystic fibrosis in the lung, metabolic diseases, and cancer. Viruses are useful here because they have in many cases evolved highly effective ways to deliver their foreign genetic information into receptive host cells. For example, common cold viruses that efficiently infect the lungs can be altered to enable therapeutic gene delivery to lung cells. Challenges await at multiple levels, spanning from the design of molecules and cells to industrial-scale processes.[1] Recombinant DNA technologies are used to design viruses that deliver the therapeutic gene, but lack the ability to reproduce or cause disease. Living cells serve as catalysts for the process, and they must also be designed to efficiently synthesize the desired virus product, ideally from chemically well-defined growth media. Finally, cell growth and virus production must then be scaled-up along with down-stream processes for product recovery, purification and formulation for clinical applications. Virus-based products are intrinsically complex, subject to biological change, and, therefore, challenging to precisely characterize.[2] As a result, both the virus products and their supporting manufacturing processes must meet the

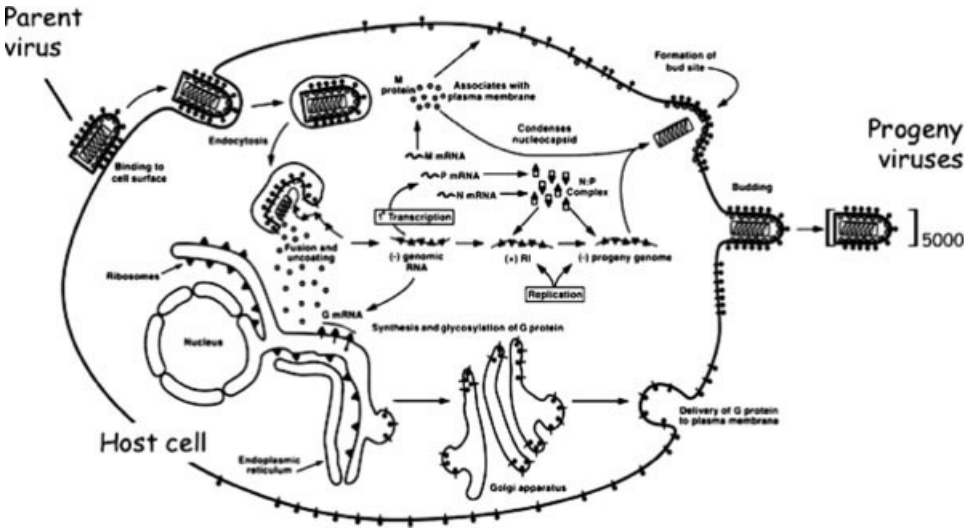

Figure 1. Virus growth: from one generation to the next.

A rabies-like parent virus attaches to a living host cell, enters, and uses cellular resources to produce 5,000 progeny virus particles that are then released from the cell. Adapted from Rose et al.[41]

challenges of the most stringent government regulatory requirements for quality, reproducibility and safety.[3]

In addition to advancing process design for the large-scale production of viruses, opportunities await chemical engineers to contribute to diverse facets of viral-based gene therapies. For example, to facilitate virus-mediated gene transfers to cells outside of the body common limitations in diffusive transport of virus particles to cells have been overcome by enabling convective transport of viruses to cells.[4] Addition of polymers has also enhanced productive virus-cell interactions through mechanisms of charge shielding and virus aggregation.[5] Quantitative modeling of virus processes within the cell have progressed from early lumped models to ones that account for transport mechanisms and spatial structure within the cell.[6] Finally, molecular design of virus particles, chiefly by genetic engineering of their surface proteins, has enabled control over the targeting of viral delivery to different cell types.[7]

Detection and characterization of viruses

The emergence of new viruses poses potential new threats to human health. Recent years have witnessed, for example, emergence of the virus that causes severe acute respiratory distress syndrome (SARS) in China, an influenza virus that transferred from chickens and ducks to humans in Vietnam, and in the U.S. the West Nile virus, which causes inflammation of the brain in humans and horses. The natural emergence of new viruses, combined with potential terrorist use of viruses as biological weapons, provides strong motivation to develop new technologies to detect and characterize viruses from patient, culture or environmental samples. Traditional approaches have focused on detection of virus nucleic acids or proteins. Currently, the most widespread methods are based on specifically amplifying viral nucleic acids by the polymerase chain reaction (PCR). If the virus carries an RNA genome, then it is initially "rewritten" in DNA form using a specialized enzyme and monomers. The PCR procedure employs thermostable enzymes, an excess of "starter" templates, monomers, and a protocol of temperature cycling that enables exponential amplification and subsequent detection of the amplified DNA. New approaches are being developed to implement amplification and detection of nucleic acids in microfluidic devices or in "lab-on-a-chip" formats, offering potentially faster and more reproducible results.[8] To address the challenge of virus detection, microarray technologies, which have been widely used to characterize active genes in a cells have been effectively adapted to simultaneously test for hundreds of different virus strains, based on sequence-specific recognition of large segments of their genomic information.[9]

Still greater challenges may await the development of "faster, cheaper, better" assays for the biological characterization of viruses. The well-established "gold-standard" for virus isolation and characterization is to show that the virus is biologically functional: the virus can infect living cells and reproduce. Diagnostic tests then focus on reproducible conditions for culture of host cells, exposure of virus to cells, and extracting quantitative readouts of the infection processes. Readouts can employ PCR or antibody-based fluorescent labeling to detect viral nucleic acids or proteins. Alternatively, treating samples with dyes that label living, but not dead cells, provides a means to visualize the effects of virus infection on cell health. Infection tests typically provide information at a single time point following exposure of virus to cells. We have extended such approaches by tracking the spatial spread of infections over multiple time points, an approach that is neither faster nor cheaper than current methods, but one that offers a more information-rich output. Initially, we implemented these methods by allowing added virus to infect a small region of a uniform film or monolayer of cells, limiting the transport of progeny viruses from infected to healthy cells by requiring that they diffuse through a gel-like (agar) matrix. Under such conditions infections can establish a constant velocity of spread, where this velocity reflects the coupling of virus reproduction with transport processes.[10] We showed how the changes in the infection velocity correlated with processes of virus evolution,[11] and activation of cell-cell communication between infected and healthy

cells.[12] Interestingly, different temporal stages of the infection process appear to spatially segregate across vast expanses of the infected cells.[13] This observation suggests prospects for elucidating the dynamics of virus-cell interactions by labeling and imaging spatial patterns of gene expression as infections spread. Recently we have replaced the agar matrix with liquid media and found that spontaneous flows enhance the spread of infection from single infected cells. We further found that by quantifying the degree of flow-enhanced infection spread we could measure the effects of anti-viral drugs with significantly higher sensitivity than current methods.[14] Given the central role of cell culture methods in the biological characterization of viruses,[15] together with recent implementations of controlled cell culture in microfluidic devices[16] there is a clear emerging opportunity for quantitative high-throughput biological infection assays in microfluidic devices.

Although virology has historically emphasized the study of infectious virus particles, noninfectious virus-like particles often accompany the cellular production of viruses. Virus-like particles arise in natural infections, as well as in laboratory and industrial-scale virus cultures. They may be chemically and physically indistinguishable from infectious virus particles, or they may exist as truncated or fragmented particles or as particle aggregates. Virus-like particles can in some cases exceed the level of infectious particles by two-or three-orders of magnitude. It is important to characterize and understand them better because they can interfere with the growth of infectious virus, they contaminate samples of infectious virus, and they can elicit an immune response, even without causing infection. Moreover, when two or more virus-like particles enter the same cell they can in some cases compensate for each other's defects, producing infectious virus progeny. Traditional measures of virus-like particles typically entail particle purification by sedimentation through a sucrose or cesium chloride gradient, electron microscopy of a fixed volume, and manual counting of particles distributed across a gird. Less labor-intensive methods of particle quantification would enable better characterization and control of virus-like particles. Potentially promising approaches include detection of fluorescently-labeled viruses or virus-like particles in flow,[17] optical detection of viruses by liquid-crystal sensing,[18] and detection of virus-specific binding to and release from a crystal microbalance.[19]

Virus-based materials

Viruses may serve as templates for the synthesis of new nanometer-scale materials. Before we consider examples, let's first explore how nature solved an interesting problem of genome packaging. Consider dimensions of various parts of MS2, a virus that infects bacteria. In Figure 2a we see that the RNA genome of this virus, which encodes four genes,

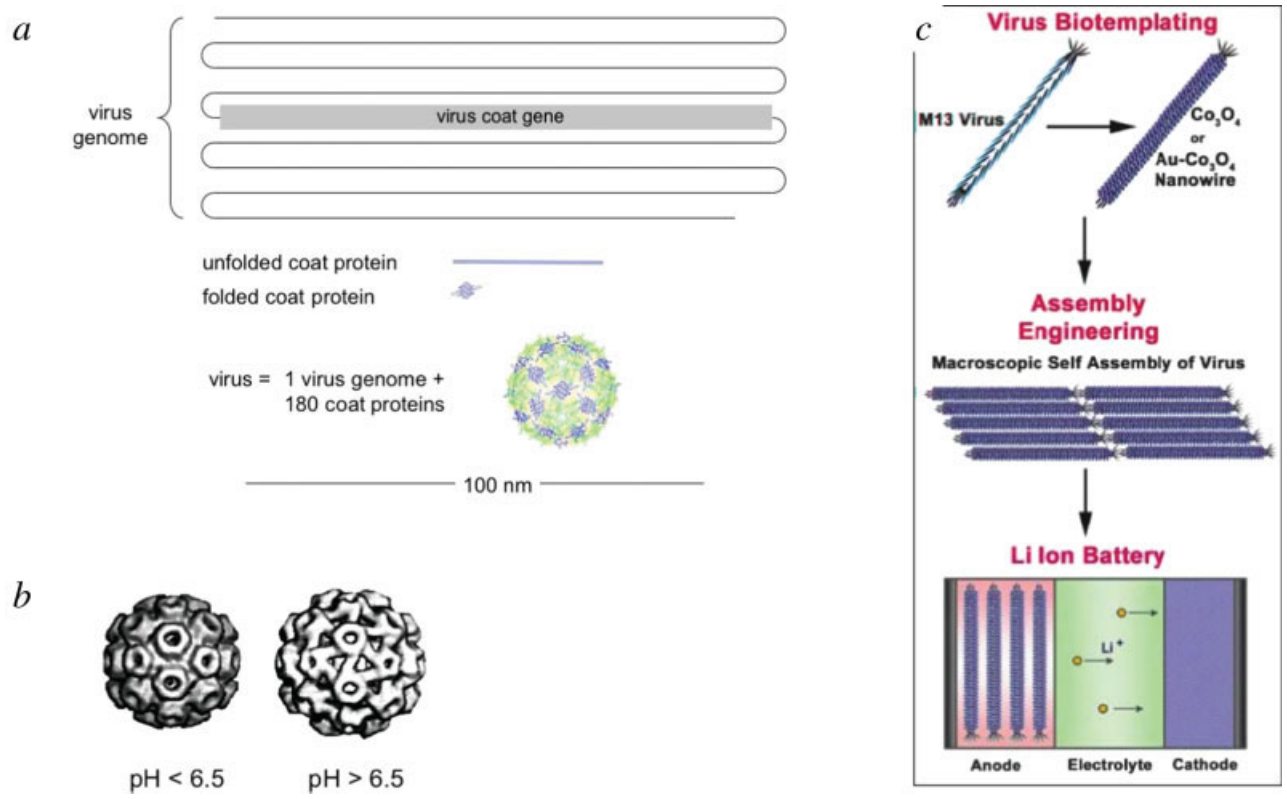

Figure 2. Viruses as templates for new materials.

(a) How a virus genome encodes its container. The virus genome encodes a coat protein that is much smaller than the genome. Many copies of the coat protein self-assemble to produce a shell that can fully package the virus genome, (b) virus structure depends on environment. Increasing the pH above 6.5 causes the virus particle to expand, enabling access to a nanoscale reaction vessel,[22] and (c) engineering of virus surfaces for new materials. Mutations introduced into the coat protein of a virus enable the surface of the virus to bind a metal oxide, producing a material with enhanced charge storage capabilities.[23]

would extend to about 1,100 nm if fully stretched out. The RNA that encodes the virus coat is only about 120 nm long, and when processed in the cell, this RNA would be used as a template to make the coat protein of the virus, which fully extended would be about 30 nm long. When folded the coat protein has a diameter of only about 5 nm. Outside the cell the virus genome would rapidly degrade if it were not protected by its protein overcoat. Yet, at 5 nm the viral coat protein is many orders of magnitude smaller than the 1,100 nm genome of the virus. So there's a problem: how could the coat protein possibly protect the entire genome? Viruses solve this problem by making many more coat proteins than genomes, and by allowing interactions between these proteins to drive multicomponent self-assembly of a shell that has sufficient volume to fully encase a packed-up genome; how the genome actually gets packaged is an unsolved problem that is motivating new research directions in polymer physics.[20] The assembly of shells from local interactions between the same or similar coat protein molecules produces the highly symmetric structures that are characteristic of many viruses. In the case of MS2 about 180 such coat proteins compose the protective shell. Biochemical and structural characterization of virus particles and their proteins, typically by X-ray crystallography and cryoelectron microscopy, allow one to anticipate how random or engineered mutations in the virus coat gene map onto changes in the chemistry of the inner or outer surfaces of the shell. The diverse shapes and sizes of different viruses, as well as the availability of biological and chemical tools to modify their protein coats, are serving expanding applications in nanoparticle design and synthesis, as reviewed elsewhere.[21] For example, a 28-nm outer diameter protein shell from a virus that infects cowpea plants has served as a nanoscale reaction vessel for the formation of single crystals of paratungstate ($H_2W_{12}O_{42}^{10-}$) from dissolved tungstate (WO_4^{2-}).[22] The synthesis was enabled by the sensitivity of the shell structure to pH (Figure 2b). As the pH of the aqueous environment is brought above pH 6.5 the structure of the shell changes from a closed to open one. The open or porous form of the shell allows release and removal of the virus genome, as well as entry of dissolved tungstate into the positively-charged cavity. Then lowering of the pH has the dual effect of nucleating oligomerization of the tungstate as the shell switches back to its closed conformation. Challenges in such approaches to materials synthesis are to identify or develop conditions that are suitable for maintaining the desired characteristics of the biological substrate and for synthesis of the inorganic material. If the virus shell is not ideal, it can be altered by introducing appropriate mutations into the gene encoding the coat protein. For example, in appropriate environments filamentous virus particles can self-assemble into dense arrays, a potentially useful characteristic for nanostructured materials. However, their surface chemistries are not well suited for interacting with nanocrystalline oxides, such as cobalt oxide (Co_3O_4), which has desirable electrochemical properties. To address this limitation, interactions between virus particles and cobalt oxide were facilitated by introducing defined mutations in the gene that encodes the major coat protein of a filamentous virus.[23] These mutations provided new carboxylic acid groups through the addition of four copies of the amino acid glutamate. Because the virus surface is composed of approximately 2,700 copies of the major coat protein, the effect of mutating the coat gene was to add about 11,000 ($= 2700 \times 4$) additional negative charges to the surface of each approximately 900-nm long virus particle. The engineered virus was then able to bind cobalt oxide, creating a material with enhanced capacity for charge storage, with potential applications in batteries (Figure 2c). The challenge to this kind of approach is to identify amino-acid combinations that will confer the desired function to the coat protein (e.g., binding of metal oxide), while still permitting the protein to create viable virus particles.

Growth and spread of viruses

Although viruses are small, their impact can be global. To appreciate this idea, consider how virus growth and spread spans many scales, as shown in Figure 3. Over short length and timescales a virus particle adsorbs to the surface of a susceptible living cell. Here the virus and cell are on the order of 100 nanometers and 10 microns in diameter, respectively. After binding to receptor molecules on the surface of the cell, a virus particle transports its genome into the cell, creating an infected cell, and, thereby, initiates a process that hijacks the cell's resources to produce typically hundreds to thousands of progeny virus particles. Release of these progeny viruses, often coinciding with death of the infected host cell, allows the viruses to spread to and infect other susceptible cells. Multiple cycles of virus amplification couple with infection spread across tissues. Different viruses tend to infect different tissues: the hepatitis viruses target the liver, notorious "cruise-ship" viruses attack the intestines, and respiratory or common-cold viruses infect the lungs. This spread of infection, coupled with cell—and tissue-level responses of the human host, define the basic processes by which viruses cause disease. Viral diseases can spread from infected to susceptible humans through direct physical contact or sharing a home or workspace. Finally, infected individuals who travel may spread viral diseases to susceptible populations on other continents.

To gain an intuitive appreciation for the explosive nature of virus growth, consider a quick back-of-the-envelope calculation. Assume that a single cell (with volume 10^{-15} m^3) infected by a single virus particle produces 100 virus particles in one day; these values are well within the productivity of known viruses. The total amount of living matter on Earth is hard to know, so we set an extreme upper bound by assuming our planet's surface has a 1 kilometer thickness of cell-like matter, giving a total volume of 10^{18} m^3 or 10^{33} cell equivalents. If every virus particle released by an infected cell immediately found and infected a new host cell, then only 16.5 generations or about two weeks would be sufficient for the descendents of a single virus particle to infect all living matter on the planet.

Of course, this calculation is wrong—profoundly wrong. A single virus particle could never have such a devastating effect. However, by considering the diverse ways the assumptions go wrong we may begin to appreciate where the greatest gaps in our knowledge reside and perhaps reveal opportunities where an infusion of new tools or methods could have impact. From an epidemiological perspective the calculation is wrong because living matter is not well-mixed.

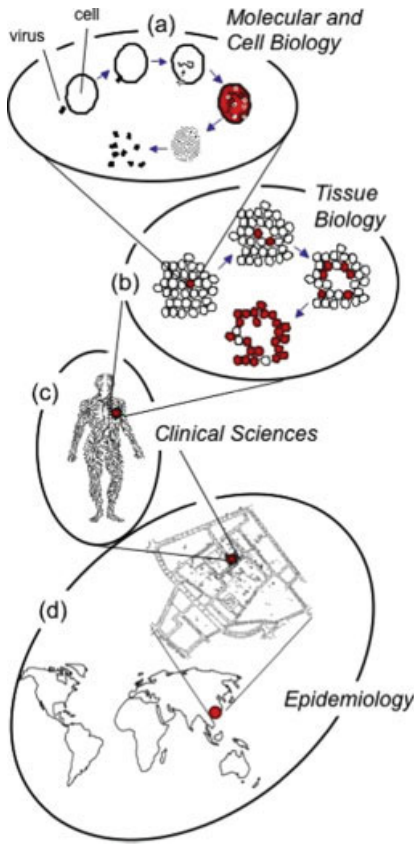

Figure 3. Virus infections span multiple scales.

(a) Over short length and timescales a virus particle adsorbs to the surface of a susceptible living cell, transports its genome into the cell, creating an infected cell (shown in red), and, thereby, initiates exploited the cell to produce progeny virus particles. Release of these progeny viruses brings about infection of other susceptible cells, (b) multiple cycles of virus amplification couple with infection spread across tissues, (c) cell- and tissue-level destruction and responses of the host contribute to viral disease. Infections also trigger host immune responses that can provide protection against subsequent infection, and (d) viral diseases can spread from infected to susceptible humans. Infected individuals who travel may spread viral diseases to susceptible populations on other continents.

Instead, people, birds, fish, microbes and plants distribute themselves spatially across the planet, richer in some locations, poorer in others. Virus progeny from one host cell do not have immediate access to all other host cells. Instead, they are limited by their ability to move. The physical movements of their infected hosts most plausibly explain how viral diseases move between continents. Hosts may be humans, mosquitoes or plants on a transcontinental airplane flight, or perhaps self-propelled migratory birds. On a more local scale, imaginable mechanisms of virus transmission between different hosts, such as human and animal, are more varied. For example, a mosquito may transfer a virus by picking it up during a blood meal on an infected animal and passing it along during a later meal on a human. An infected human may transmit virus to a susceptible human through a transfer of bodily fluids: through a sneeze, cough, sexual act or handshake. Our calculation also fails because humans are not well-mixed. The virus released from a single infected cell within our body does not gain immediate access to all other cells. Susceptible cells for subsequent infection may be accessible by physical processes of diffusion to nearby cells or by convective flow in blood or other fluids to distant cells. What we may begin to appreciate here is that the growth and spread of viruses depends not only on the biological process of cell infection, but also on the physical transport of viruses or infected cells in fluids or aerosols.

The calculation is also wrong for many biological reasons. Here we highlight three. First, viruses tend to be selective in the kinds of cells they infect. All viruses need to exploit the protein-synthesizing machinery of living cells to grow, and all living cells possess this machinery. However, no known virus is able to infect all living cells. Viruses that infect bacteria do not infect animals, and viruses that infect plants cannot infect humans. However, viruses that infect animals can in some instances infect humans. Cells within multicellular organisms like humans tend to coordinate their functions with other cells. They communicate using molecular signals that are synthesized and released by cells with information to convey and received by the surface receptor molecules of cells seeking updates on their environmental conditions. The different functions of different cells, whether they are heart, lung or brain cells, tend to correlate with different kinds and distributions of surface receptors. Viruses recognize and infect cells based on the specificity of surface receptors they encounter. The receptors on cells of our intestines tend to differ from the receptors on cells of our lungs, so viruses that cause diarrhea tend to differ from viruses that cause respiratory infections. Moreover, viruses mutate as they grow, providing opportunities for their receptor preferences to change. There is current concern that stains of influenza A virus in birds may chance upon the combination of mutations that enable them to become a deadly virus in humans. Returning to our quick calculation, it is implausible that any single virus could universally infect the diverse forms of life on our planet.

Our explosive infection scenario is implausible for a second biological reason. Organisms are seldom the fully passive prey to virus infections that we suggested in the example. Instead, living organisms, from the simplest bacteria to humans, mount defensive responses to infections by viruses. Bacteria produce enzymes that can recognize and inactivate the DNA of invading viruses. Upon infection, cells in our body can set up innate defensive responses, producing molecular signals that spread to and warn other cells of their encounter with virus. Upon receiving such warnings, cells that are susceptible to infection can shut down the biosynthetic functions that are essential for virus growth, effectively limiting spread of the infection. Systems of highly coordinated cells can also develop an adaptive immune response that specifically targets viruses and infected cells for destruction. It is this adaptive response that we stimulate when we are vaccinated. Finally, our explosive infection example neglects the roles of evolutionary and ecological forces in shaping the behavior of virus infections. Parasites depend on their hosts for their own reproduction, as well as transmission to new hosts. Highly virulent viruses reproduce efficiently at the expense of their hosts. By killing off hosts too efficiently, they potentially reduce interactions between infected and

susceptible hosts and thereby limit their own spread. As a consequence of such mechanisms, viruses that persist in nature tend to evolve relationships that also allow their hosts to persist.

Models of virus growth and spread

To gain insight into the behavior of viruses and viral diseases, models have been developed with an aim to elucidate underlying mechanisms. To better understand how viral and other infectious diseases spread through susceptible populations, epidemiologists have historically developed models that account for interactions among susceptible, infected and recovered hosts. These are essentially mass-action models that allow collisions between susceptible and infected individuals to produce more infected individuals who then pass on the infection, recover or die. Such models have been extended to allow for spatial variation through the inclusion of diffusional transport of its hosts, and recent trends in epidemiological modeling are increasingly structured, incorporating census data with the simulated behavior of social and transportation networks. In the last decade ecologists have advanced predator-prey models that have played an important role in understanding the dynamics of virus infections within individual patients.[24,25] These models have provided a means to interpret clinical data on how antiviral drug treatments and host immune responses affect virus levels and disease progression. Here a major challenge is to understand how recognition (and suppression) of the virus by the immune system tracks with rapid genetic changes that occur within a patient's virus population. Recent intriguing models based on principles of statistical physics suggest that diverse viruses share a common strategy of creating self-competition within the host immune response, a process that the viruses then exploit to persist.[26] Such models then also suggest alternative strategies for vaccination. At a still higher resolution, experiments at the molecular and cellular level are revealing how physical contacts between cells define the earliest stages of immune cell activation. Notably, reaction-transport models have provided a useful framework for understanding how multiple membrane-associated components organize in space and time.[27] At this level of molecules we and others have worked to develop models of virus growth within cells.[28–30]

From a chemical engineering perspective, one may view virus growth as a process that converts raw materials and resources of the living cell into virus product. The process has a well-defined beginning, corresponding to the binding of a virus particle to the surface of a host cell, and a relatively well-defined end, where virus progeny are released by the infected cell into the environment. Models of virus growth within cells aim to account for the essential processes that occur between these events. One typically initiates model development by assuming the essential resources of the cell are available in excess. This assumption implies that the dynamics of virus development is primarily defined by the "decoding" of the virus genome. A basic overview of how genomic information is chemically encoded and decoded is available elsewhere.[31] Inevitably, interactions among virus and cell components define positive and negative feedback loops that regulate the utilization of resources. More refined models that account for finite cellular resources have, together with experiments, highlighted both the central and expensive role of protein synthesis in virus growth.[30,32] Models of virus growth have also enabled a better understanding of how the many separate components interact to yield behavior that is robust to perturbations in virus or cell functions.[33,34] Moreover, models may also serve as useful tools for designing safe live-virus vaccines. Such vaccines, which rely on the weakened or attenuated growth of a virus to stimulate an immune response, have historically been created by culture methods that bring about a loss in viral virulence owing to poorly understood mechanisms. Today, with the availability of tools to create genetically altered viruses, there is hope of designing vaccines that perform with a controlled degree of growth attenuation. In cases where the timing and level of synthesis of virus components depends on the genome organization of the virus one may re-order the genes, and, thereby, alter the growth.[35,36] In this context models serve at a tool to codify the known interactions among virus, and host components and to predict how re-ordering their timing and levels will affect virus growth. Finally, efforts to develop antiviral drugs target virus functions with the aim of shutting down growth. Sensitivity analysis of virus models may offer opportunities to identify an "Achilles heel" for virus growth before one sets foot in the laboratory. Moreover, virus models can be used to explore how viruses develop resistance against drugs and suggest counter-strategies that resist escape.[37,38]

Many stimulating challenges and opportunities await those seeking to better understand how viruses grow and spread. At the molecular and cellular levels the details of how viruses enter cells and unpack their genomes, how they assemble, and how they are released from the cell generally lag behind our understanding of viral gene regulation. Moreover, because a single virus particle carrying a single DNA or RNA genome is sufficient to initiate an infection, fluctuations in molecular levels may significantly contribute to distribution of outcomes across a population of infected cells, motivating the development of stochastic models of virus growth.[39,40] Perhaps the greatest challenges will be to link "bottom-up" single-cycle models of virus growth with "top-down" models,[24,25] incorporating the effects of a patient's immune defenses on his or her virus load. Such models would ideally describe how mechanisms of virus growth, spread and immune activation influence how viruses cause disease, and, thereby, provide a foundation for tailoring therapeutic care to the needs of the patient.

In summary, we have sought to highlight how four facets of virology create new research challenges and opportunities in chemical engineering. First, as products that promote human health, virus-based vaccines and gene-therapy agents have the potential to save millions of lives. At the same time, they define the most complex products and highly regulated manufacturing processes in the pharmaceutical industry. Second, tools and methods to characterize and quantify the physical, chemical and biological properties of viruses remain labor intensive and limited. Advances on this front will significantly impact both fundamental and applied virology research. Third, viruses offer a remarkable array of structurally defined templates for the controlled synthesis of new materials. Moreover, the ability to mutate virus genes provides unprecedented control over their resulting surface

chemistries. Finally, to understand and ultimately control how viruses grow, spread and persist is among the grand challenges of biology. Chemical engineers are uniquely qualified to make important contributions in this arena, especially in the defining and advancing quantitative and integrative methods and models.

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