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Integrating cellular and molecular structures and dynamics into whole-cell models

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Abstract

A complete description of the state of the cell requires knowledge of its size, shape, components, intracellular reactions, and interactions with its environment—all of these as a function of time and cell growth. Adding to this list is the need for theoretical models and simulations that integrate and help to interpret this daunting amount of experimental data. It seems like an overwhelming list of requirements, but progress is being made on many fronts. In this review, we discuss the current challenges and problems in obtaining sufficient information about each aspect of a dynamical whole-cell model (DWCM) for simple and well-studied bacterial systems.

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Introduction

The goal of whole-cell modeling is to generate predictions about cell behavior from cell states evolving forward in time. From a basic science perspective, such an endeavor involves the integration of a daunting amount of experimental data along with theoretical and algorithmic studies into whole-cell models (WCM) with the promise of revealing simultaneously results and correlations that will deepen our understanding of the principles of life. To describe the state of a cell, requires information about the system's initial states, the evolution rules, and a method to apply the time-evolution. In the context of WCM we refer to these as the composition, interactions, and propagation, respectively. Composition includes knowledge of the cellular architecture and the concentrations of the biomolecules and metabolites in three-dimensional space. Interactions include physical and chemical interactions among the chemical species and with the extracellular environment. Propagation refers to the necessary computational methodologies to time-evolve a given cell state according to the interactions over biologically relevant length and timescales. The existing WCMs vary in the details of composition (entire cell or just a selected subvolume of the cell), physical interactions (atomistic force-fields to crowding by excluded-volume, each resulting in a different representation of chemical species), chemical reactions (ranging from thousands of genome-wide chemical transformations to none), and propagation (molecular dynamics, stochastic diffusion and chemical kinetics, and PDEs/ODEs). With advances in biophysical experimental and computational methods, progress is being made on all fronts. In this article, we discuss the challenges and problems in obtaining sufficient information from the standpoint of our own experience in creating 3D and well-stirred dynamical whole-cell models (DWCMs) for a living minimal cell [1].

Cellular shapes and architecture

The physical and chemical methods that allow us to simulate conformational changes in biomolecules, determine functional forms of large complexes in solution, and locate labeled protein complexes and RNAs in dense media, respectively have been recognized by Chemistry Nobel Prizes for molecular dynamics (2013), cryo-electron microscopy (CEM; 2017), and super-resolved fluorescence microscopy (SRFM; 2014). These Nobel Prizes rest upon previous prizes awarded for methods of obtaining structures of biomolecules from X-ray crystallography and in solution from NMR. Recent advances in cryo-electron tomography (CET) and super-resolution imaging (SRI) have led to an increasing amount of information about the structures of single cells and the dynamics of the molecular actors within them, respectively.

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CET of entire small bacterial cells like *Spiroplasma melliferum*, *Mycoplasma pneumoniae*, and the synthetic genetically minimal cell, JCVI-syn3A, can reveal the three-dimensional cell shape and spatial distributions of ribosomes within the cell [2–4,1]. In the case of *M. pneumoniae* [3], the in-cell architecture of a transcribing and translating expressome was resolved at subnanometer resolution through crosslinking mass spectrometry, subtomogram averaging, and integrative modeling. The expressome comprises RNA polymerase (RNAP), the ribosome, and the transcription elongation factors NusG and NusA. With NusA being pinpointed at the interface between a NusG-bound elongating RNAP and the ribosome, it was proposed that the expressome can mediate transcription-translation coupling. The frequency of such coupling is still uncertain and seems to vary among bacterial species [5]. Typically, bacterial nucleoids are not directly observed in CET, but their spatial localization can be inferred from the ribosome distributions. Generating ensembles of DNA configurations consistent with the ribosome distributions and bacterial genome organization principles inferred from sequencing techniques such as chromosome conformation capture (3C) [4,6,7] and CHIP-seq [8,9] methods is necessary for whole-cell simulations [1]. Nucleotide-resolution molecular models of the condensed 4.6 mbp *Escherichia coli* chromosome [8] are presently the highest-resolution cell-scale structural models of condensed bacterial nucleoids. Dynamically altering the local DNA structure during a WCM simulation at any level approaching nucleotide representation has yet to be achieved. When numerous CET are obtained from a population of cells, then correlations between cell size and ribosome distributions can be observed, within which chromosomes at different points in replication can be placed [4].

For CET of larger bacterial and eukaryal cells, focused ion beam (FIB) milling produces lamellae a few hundred nanometers in width from a population [10–12], and in the case of HeLa cells sufficient sampling has produced invaluable information on the organelles neighboring the nucleus and the nuclear pore complexes [13]. The goal to establish visual proteomics from CET has been recently reviewed in the study by Bäuerlein et al. [14] along with the remaining technical challenges to achieve subnanometer resolution. These goals will mostly be achieved with advances in technology, but there is an additional challenge to have structures or accurate predicted structures of all the biomolecules to support integrative modeling. The interpretations of the CET and SRI processing will certainly be aided by machine learning approaches such as AlphaFold2 [15], which can utilize the large and growing data sets of known protein structures. Since its impressive performance in CASP14, it is already being leveraged to predict or complete protein structures used in molecular dynamics simulations of Cas9 proteins [16].

Concentrations of proteins, mRNAs, lipids, and metabolites that provide the initial states of a cell are typically estimated from proteomics, transcriptomics, lipidomics, and metabolomics [17] data. In our own experience, counts of membrane proteins from proteomics and the complexity of the lipid composition from lipidomics can be integrated to model an initial membrane composition [1]. Complexity of the lipidome includes the degree of saturation of the fatty acids, the number of different lipids, and dynamical information about membrane remodeling during cell growth. The composition of cell membranes not only affects the overall architecture of cells, it also affects structure, organization, and function for both RNAs [18] and proteins, where some perform their functions in the membrane periphery (ex. Gram-positive degradosomes [19]).

The remaining proteomics, transcriptomics, and metabolomics data can then provide the initial concentrations of molecules present in the cytoplasm. Unfortunately, while there are numerous -omics studies on bacterial organisms, these studies are typically limited to a few growth conditions and report quantities at the population level rather than the single-cell level. As a further complication, many of these studies do not provide an exhaustive survey of complete proteomes, transcriptomes, lipidomes, or metabolomes and report only subsets of chemical species or incomplete information. Consequently, initial states of the membrane and cytoplasm need to be prepared using datasets consolidated from multiple organisms, which frequently require scaling laws for concentrations based on cell volume and genetic lineage; akin to how homology modeling has allowed us to generalize our knowledge of protein and nucleic acid structures. Presently, organization of cytoplasmic molecules beyond compartmentalization in organelles has been treated using uniform distributions in the absence of 3D localization in the -omics data. As discussed above, 3D localization in proteomics is a future goal of CET. The resolutions of techniques for 3D single-cell transcriptomics in eukaryal cells [20] are being extended to smaller and denser biological systems [21].

Kinetic models of subcellular dynamics

Combinations of crystallographic structures and single-molecule (sm) FRET can provide mechanistic insights and assist in the development and parameterization of kinetic models for fundamental processes such as bacterial DNA replication initiation [1]. Crystal structures of DnaA bound states to double- and single-stranded DNA revealed sequence-dependent binding near the origin [22,23] and smFRET provided on/off rates of DnaA filament formation on single-stranded DNA [24]. With the limited photons emitted from fluorophore labels inside living cells, capturing subcellular dynamics is restricted by a trade-off between spatial/temporal

resolution and phototoxicity. Developments in the super resolution microscopy techniques of PALM, STORM, and STED for imaging of processes within the nucleus to obtain minimal phototoxicity was reviewed [25]. A new fluorescence microscopy technique, MINFLUX (MINimal photon FLUXes) [26,27], promises to provide a particle tracking technology with a resolution 10 times better than other single-molecule techniques such as STORM and PALM. MINFLUX uses a donut-shaped excitation beam to surround a single fluorescing molecule with light. The position of the molecule is then triangulated by moving the beam to targeted locations and minimizing the fluorescence intensity while shrinking search path of the beam. As a result of needing very few photons (800-2500) to localize the fluorophore, its 3D imaging can locate molecules to 2 nm with a tracking resolution of 100 μs while retaining the multicolor and sample preparation advantages of fluorescence microscopy. In the study by Schmidt et al. [27], the spatial-temporal resolution in single fluorophore tracking was reported for the diffusion of single labeled lipids in lipid-bilayer model membranes, and MINFLUX images of nuclear pores in living U-2 OS cells expressing Nup96−mMaple were obtained in the study by Gwosch et al.[26]. With the similar spatial and temporal resolutions of MINFLUX and whole-cell simulations [1], hopefully more examples from live cells will soon be available to make the integration of experimental kinetic data into WCMs more straightforward.

All-atom models have not yet achieved cell-scale simulations. However, models of cytoplasmic spaces, such as the 100 nm cube model of DNA-free *Mycoplasma genitalium* cytoplasm [28,29] with biologically accurate protein, RNA, metabolite, and ion concentrations, are substantial efforts that provide valuable dynamic data including diffusion coefficients, conformational changes, and protein-protein contact dynamics over tens to hundreds of nanoseconds [30].

While the majority of molecular dynamics simulations are carried out over hundreds of nanoseconds, there have been some notable achievements in extensions into the microsecond regime to examine the diffusive interactions of transcription factors along short pieces of DNA using all-atom and coarse-grained simulations [31,32]. Coarse-grained models in which approximately four heavy atoms in the proteins and lipids are represented by a single bead reduces the number of equations of motion and hold the promise of pushing simulations into more biologically relevant time scales. The Martini 2 and 3 force fields [33] have been used to model lipids of an entire mitochondrion [34] and lipids with hundreds of membrane proteins (14 types) in protocells [35]. Even though they are not all-atom, the coarse-grained simulations can still be used as an additional validation of WCMs.

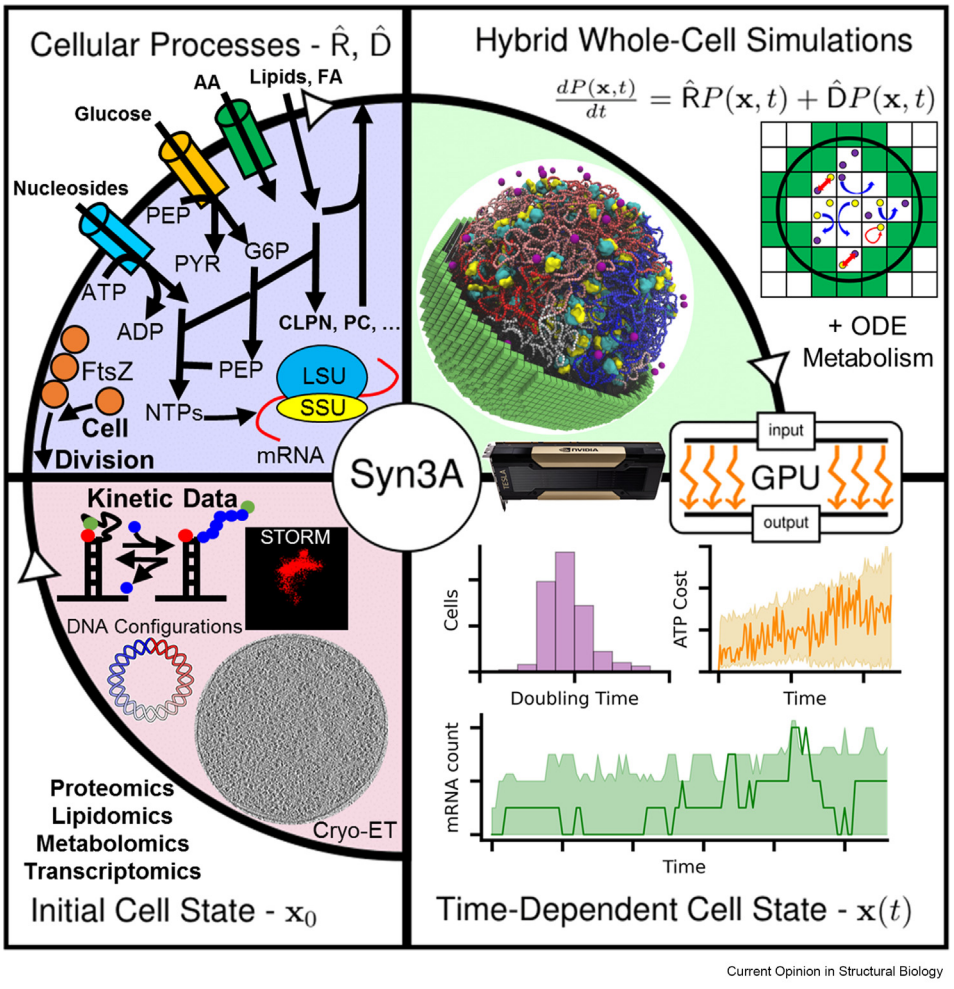
Whole-cell structure-based models that coarse-grain entire macromolecules have been created for cytoplasmic and membrane macromolecules in a *M. genitalium* cell [36] using well-stirred states generated from the WCM in the study by Karr et al. [37]. The placement of the macromolecules accounted for steric interactions and was used to test the viability of predicted cell states. The structural model of *M. genitalium* faced the further challenge of assembling a set of individual structures for the proteome. Structural models of proteins with known function were assembled using a combination of AlphaFold2 [15], the Protein Data Bank, Electron Microscopy Data Bank, UniProt, and homology modeling.

Integrating structure and reaction networks

CET provides a static cell-wide picture of large cellular structures, SRI provides single-molecule dynamics of individual molecular systems, and WCMs serve as a mean to bridge the gap between these techniques until future methods to observe more complete dynamical cell states are developed. Importantly chemical reactions associated with metabolism and information processing networks need to be included in order to capture the subcellular dynamics. The development of WCMs and how they have progressed from genome-scale metabolic models (GSMMs) [38] and the calculation of their steady-state fluxes has been recently reviewed [39,40]. Due to large numbers of genes with unknown function and the complexity in model systems such as *E. coli*, along with the broad range of length-, concentration-, and time-scales that need to be considered, simulating a complete description of the state of a cell has been challenging and requires hybrid computational methods.

Comprehensive WCMs have been developed for *M. genitalium* [37] and *E. coli* [41] where the subsystems were treated in terms of flux balance analysis, ordinary differential equations, and stochastic simulations. We recently simulated a synthetic minimal cell consisting of nearly exclusively the genes and reactions necessary for the cell to proliferate in a laboratory environment and we believe this is an ideal system for a WCM [1,42]. JCVI-syn3A is a living genetically minimal bacterial cell with a chemically synthesized genome consisting of only 493 genes on a 543 kbp chromosome, 452 of these genes are protein-coding [43]. Syn3A's genome and physical size are approximately one-tenth those of the model bacterial organism *E. coli*. The simulations of this organism in the study by Thornburg et al. [1] are based on fully dynamical kinetic models where subsystem networks and chemical species are interconnected continuously over time on a single-cell basis (Figure 1). The 3D spatial models are reconstructed at the single-cell level using ribosomes coordinates from CET and self- and ribosome-avoiding DNA configurations. Common challenges among these whole-cell modeling efforts are

Figure 1



Diverse experimental techniques and data characterizing the cellular state and processes of Syn3A are integrated into a DWCM for simulations of time-dependent cell states. Ribosomes were placed in the 3D spatial cell model using PDB 5NJT and the instancing visualization method in the study by Sener et al. [54] (Adapted from the study by Thornburg et al. [1] and includes unpublished data.).

characterizing cell architecture, establishing the reaction networks, and the limited availability of kinetic parameters and -omics data to use as initial conditions. No one bacterium has a complete set of structures, parameters, -omics data, and annotated genes with known functions. The development of any WCM relies upon synthesizing information from other organisms.

The *M. genitalium* [37] and *E. coli* [41] models tackled the challenge of curating large datasets consisting of thousands of kinetic parameters and demonstrated methods of evaluating parameters from other species and the variations that have been measured in even a single species. In our own WCM of Syn3A, initial estimates for every kinetic parameter and metabolite concentration in the metabolic reactions were determined [1] using a Bayesian estimator [44] with prior information consisting of a survey of concentrations from *E. coli* [17] and *M. pneumoniae* [45] and parameters measured in related organisms through decades of biochemical, smFRET, and spectroscopic studies reported in the literature and kinetic databases like [46], BRENDA [47], and equilibrium constants reported in NIST's TECRdb [48] and Equilibrator [49]. Each of the WCMs discussed has taken a different approach to utilizing the vast amounts of available kinetic, biochemical, and structural data. While there is not yet a clear answer to the best method of curation, the systematic organization and reporting of these parameters and structures in federated and connected databases will continue to be critical for assembling WCMs that provide unified descriptions of cell behaviors.

Simulation methodologies

Simulation methodologies for cellular processes are reviewed in the study by Smith et al. [50] so we provide only a brief summary here. In the case of our DWCM of a living minimal cell, we employ hybrid multiscale methods to propagate the cell state [1]. At the greatest level of complexity in time- and length-scales, intracellular kinetics in a three-dimensional and spatially heterogeneous environment are modeled by the reaction-diffusion master equation (RDME) approach, where particles stochastically diffuse between and react within subvolumes. For the homogeneous case in which the particles are considered to be well-stirred due to fast diffusion, the chemical master equation (CME) is used to describe stochastic reactions. In both cases, the particles are assumed to be in rather low concentrations. And in the limits of high concentrations and fast diffusion typically associated with metabolites in metabolic reactions, the reactions are described with ordinary differential equations (ODE). Given the current state of the art in whole-cell modeling, we feel our approach to WCMs remains faithful to the known interactions within the genome-wide biochemical reaction networks, while resolving the spatial heterogeneity within the cell (positions of ribosomes, circular DNA, and membrane) and enabling simulations over portions of the entire cell cycle. However, this choice in methodology assumes simple physical interactions between the particles and crowding, and an important future step includes the backtransformation for the state of a cell in such a particle picture to one that includes either all atom or coarse-grained structures of the cytoplasmic and membrane species, see Figure 1.

Concluding remarks

The DWCM in the study by Thornburg et al. [1] presented a three-dimensional model of a living minimal cell Syn3A, and used (RDME/CME/ODE) simulations to study cell behavior over 20 min before any substantial cell growth or DNA replication had occurred. From these relatively short runs, probabilistic factors were estimated that allowed predictions about responses to the environment, complexifying pathways and time correlations between the cellular processes of DNA replication and cell growth over the full cell cycle using well-stirred approximations. Determining the balance of energy generation and costs for each process was an important first step to measure certain metabolites and cellular intermediates as a function of time. As more experiments and theoretical models on DNA replication and segregation become available, more complex growth behavior in three-dimensions can be addressed over the entire cell cycle. Because of the large variation in time-scales and concentrations, the three-dimensional DWCM for Syn3A required combined hybrid stochastic-deterministic simulations using the GPU-based simulation software Lattice Microbes [51,1]. GPU computer clusters represent yet another necessary revolution to bring structural biology and its wealth of information to understanding how even a minimal cell functions.

We identify two complementary directions to further advance whole-cell modeling: aspects of unknown compositions and interactions in cells must be addressed by experimental methods and computational modelers must develop novel techniques to accommodate more complex compositions and interactions. For example, MEDYAN was recently extended to include mechanochemical interactions between the cytoskeleton and membranes [52]. No organism has a gene set where every function is known. While the number of genomes being sequenced has increased, the substantial fraction of genes with products of unknown functions (for example, roughly 40% in *E. coli* [43]) leaves gaps in models and limits their predictive power by leaving out possible chemical and physical interactions. Among the genes in Syn3A, there are seven genes known to be necessary to maintain regular spherical morphology upon cell division, but their exact functions are unknown [53]. Determining structures and performing functional kinetic studies on such genes is going to be critical for the construction of complete WCMs. In general, with the increasing abundance of dynamic and structural data of large-scale reaction networks, a more systematic approach is needed to determine or estimate the organism-specific kinetic parameters.

Conflict of interest statement

The authors declare no conflict of interest.

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