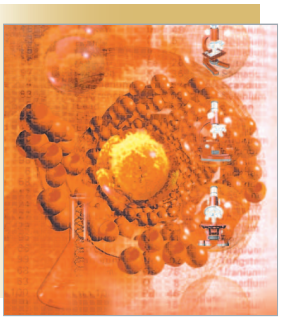


Deriving Principles of Microbiology by Multiscaling Laws of Molecular Physics

Applications in Nanomedicine and Energy

BY PETER J. ORTOLEVA, P. ADHANGALE, S. CHELUVARAJA, MAX W. A. FONTUS, AND ZEINA SHREIF

It has long been an objective of the physical sciences to derive principles of biology from the laws of physics. At the angstrom scale for processes evolving on timescales of 10^{-14} s, many systems can be characterized in terms of atomic vibrations and collisions. In contrast, biological systems display dramatic transformations including self-assembly and reorganization from one cell phenotype to another as the microenvironment changes. We have developed a framework for understanding the emergence of living systems from the underlying atomic chaos.

Our conceptual starting point is the recognition of the key role of coupling among processes across many scales in space and time that underlies microbial systems (Figure 1). Highly fluctuating atomic-scale processes lead to the thermal-average and frictional forces driving the evolution of order parameter (Figure 2). In our studies, order parameters characterize suprananometer-scale features of a virus, nanocapsule, or other microbiological or nanomedical structures. However, these order parameters control the statistics of atomistic fluctuations. This completes a key feedback loop underlying the microbial behavior. This feedback is ignored in decoupled coarse-grained theories [1], [2].

The significance of the workflow of our project (Figure 3) is both fundamental and practical. It is fundamental because it advances and integrates methods in the theory of Brownian motion to understand the stochastic world of nanobiology. It is practical since it can lead to calibration-free models of microbes and nanomedical systems that can, for example, serve as the basis of computer-aided vaccine or microbial fuel cell design strategies.

Phenomena under study in our center include viral structural transitions, bionanosystem (BNS) migration and self-assembly, and self-organized chromosome segregation and division in bacteria. In this article, we provide more details on the conceptual flow (Figure 2) and on our applications to specific biological phenomena. Implications for the medical and energy sciences and conclusions are drawn.

All-Atom Multiscale Analysis of BNSs

To derive principles of microbiology, we assume that the N-atom system evolves via classical mechanics. Thus, our starting point is the application of Newton's laws to molecular systems. These laws describe the dynamics of the N-atom configuration of the virus, ribosome, bacterium, or other microbiological structure. As the laws of molecular physics are well established, this provides a reliable starting point. However, present-day molecular dynamics (MD) is not practical for simulating these supramillion atom systems by a direct all-atom approach. For example, the highly optimized MD package nano molecular dynamics (NAMD), run on a 1,024 processor platform, would take about 3,000 years to simulate a structural transition in a small virus over its characteristic millisecond timescale. The object of our approach is to use the multiscale character of BNS to overcome this barrier and enable the computer simulations needed to discover the principles of microbiology.

There have been other attempts to overcome the computational barrier, but they have limitations. Decoupled coarse-grained MD [1], [2] groups atoms into lumped elements and calibrates effective forces between them. This misses the feedback given in Figure 1 and makes the incorrect assumption that the lumped elements satisfy simplified laws (Langevin equations). However, a more rigorous multiscale approach shows that if the lumped elements are too small they satisfy friction-dominated, non-Newtonian mechanics; if they are too large, then the exchange of energy between their internal degrees of freedom and those of neighboring lumped elements must be accounted for via the feedback shown in Figure 1. In contrast, a rigorous way to reduce the computation is to use projection operators [3]. However, this introduces factors into the reduced set of equations for the dynamics of the multiatom features of interest; unfortunately, these factors are difficult to evaluate in terms of the interatomic forces or via MD.

In contrast to the all-atom starting point we advocate, one usually conceives biology in terms of nanoscale or larger components. A virus contains several hundred protein units (capsomers) that encase the genetic material and, for enveloped viruses, contains an outer membrane. The dynamics of a virus is described by virologists in terms of these subunits. However, it is not always clear if these units are well defined (e.g., capsid pentamers or hexamers) or are simply expressions of icosahedral symmetry and are not well defined energetically, i.e., are not invariant over the process of interest.

What is needed is a set of variables that can be automatically constructed and which do not impose preconceived notions of structure and dynamics. Such an approach would

Digital Object Identifier 10.1109/MEMB.2009.932389

 0739-5175/09/$25.00©2009IEEE

Biological systems display dramatic transformations including self-assembly and reorganization from one cell phenotype to another as the microenvironment changes.

avoid omitting unforeseen structures or transition pathways connecting them. Variables characterizing the supramillion atom-scale features that we use in our studies are of two types. The first type describes objects that are intact during the process of interest, e.g., macromolecules during viral self-assembly; these are generated by conceiving all space as a deformable medium, embedding the system in this space and introducing order parameter Φ that generate the transformation of interest via deformation of space. Example processes that can be treated in this way are viral capsid structural transitions or bacterial daughter chromosome segregation following replication. The second type describes interdiffusing molecular species and includes generalized density variables. These variables are much like molar concentrations and describe the spatial distribution of a cloud of many individual features (e.g., drug molecules escaping from a nanocapsule delivery system or phospholipids in a membrane). For the intact system components, we introduce a discrete set of order parameters. However, for interdiffusional phenomena, we introduce an uncountable infinity of variables, i.e., one for each of the uncountable set of spatial points within the system [4].

To proceed, the order parameters must be related to the all-atom configuration. With this, we determine, via Newton's equations, if the order parameters evolve slowly relative to the timescales of atomic vibrations or collisions. When this timescale separation is present, a factor ε, the ratio of the characteristic time of atomic dynamics to that of order parameter evolution emerges. For phenomena involving the rearrangement or assembly of major viral components, this can be 10^{-5}. Thus, if one could derive equations for order parameter dynamics free of explicit atomistic fluctuations, they would evolve roughly 10^{-5} times slower than atomic vibration. This implies that a simulator based on such equations could proceed 10^5 times faster than MD, without loss of accuracy or unjustified simplification. Such an approach avoids the need for recalibration with each new application, as it is based on the laws of molecular physics. Thus, simulation of a BNS over biologically relevant time periods would then be feasible.

Our procedure is outlined and reviewed earlier in Figure 2. The system is described via the positions and momenta Γ of the N atoms (a total of $6N$ variables). As we are not able to comprehend or are interested in the detailed time course of each atom, we introduce the probability density ρ for the state Γ at time t. The evolution of ρ is determined by the Liouville equation. To evoke the biology from the atomistic chaos described by this equation, we make the hypothesis that ρ depends on Γ both directly and, via our Γ-dependent order parameters, indirectly. The system, and hence ρ, evolves on multiple timescales, i.e., 10^{-14} s for atomic vibrations to

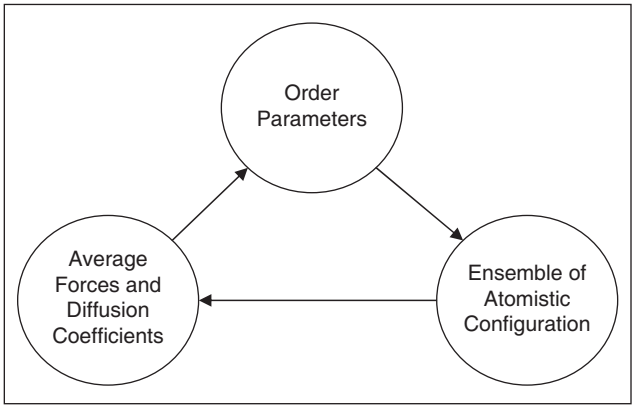


Fig. 1. Order parameters characterizing nanoscale features affect the relative probability of the atomistic configurations, which in turn, mediate the forces driving order parameter dynamics. This feedback is central to a complete understanding of nanosystems and the true nature of their dynamics. Our rigorous multiscale approach captures all such feedback by coevolving the order parameters with the thermal-average forces and coefficients quantifying the role of frictional forces.

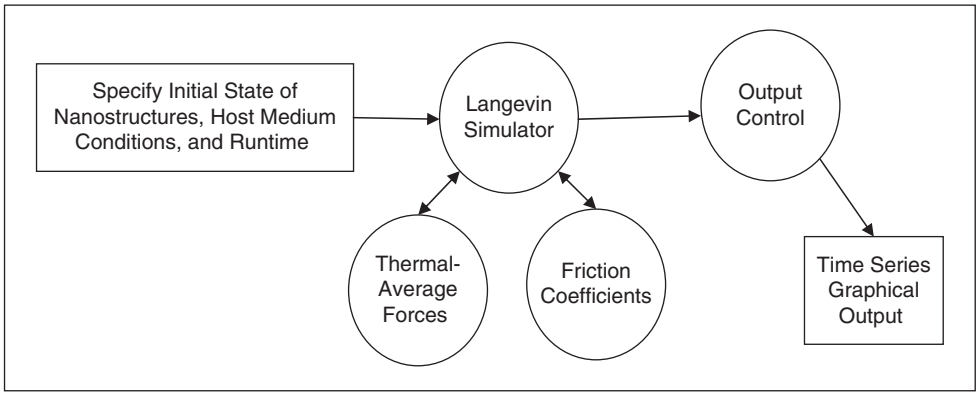


Fig. 2. Schematic multiscale algorithm indicating that thermal-average forces and friction coefficients are computed on the fly, since they coevolve with the state of the bionanosystem by solving Langevin equations. This has been implemented as a simulator NanoX, a multiscale software for modeling nanosystems.

A multiscale perspective provides insights into bioelectric phenomena and a way to solve otherwise difficult equations.

10^{-3} s or longer for viral migration or bacterial daughter chromosome segregation. Thus, we introduce a set of time variables $t_n = \varepsilon^n t,\ n = 0, 1, \ldots$ to track the distinct ways in which ρ depends on time t. Although t_0 tracks atomistic timescale events, the set of longer times $t = \{t_1, t_2, \ldots\}$ track the slower processes, i.e., t_n changes by about one unit when time t changes by ε^{-n}.

Our hypothesis on ρ takes the form

$$\rho = \rho(\Gamma, \Phi, \Psi, t_0, t; \varepsilon), \tag{1}$$

where Φ is a set of discrete order parameters, Ψ a set of spatial profiles of order parameter field variables, and t_0, t track the various dependencies on time. With this and the chain rule, the Liouville equation takes the multiscale form

$$\sum_{n=0}^{\infty} \varepsilon^n \left(\frac{\partial}{\partial t_n} - \mathsf{L}_n \right) \rho = 0, \tag{2}$$

where the L_n arises from the original Liouville equation and the chain rule. The operator L_0 drives the short timescale (t_0) dynamics, while the L_n for $n > 0$ mediates the slow evolution associated with order parameter dynamics.

To solve the aforementioned problem, we take advantage of the timescale separations, i.e., the smallness of ε. We write ρ as a Taylor series ($\rho = \rho_0 + \rho_1 \varepsilon + \cdots$), then analyze the multiscale Liouville equation order by order. To lowest order, (2) implies $\partial \rho_0 / \partial t_0 = \mathsf{L}_0 \rho_0$. For most microbial phenomena, the evolution of interest is slow (i.e., on timescales greater than a nanosecond). Thus, ρ_0 is assumed to have no t_0 dependence. On this timescale, the system explores many atomistic configurations and hence can be described via a probability distribution that coevolves with the slowly changing order parameters. In this case, we show that ρ_0 has the quasi-equilibrium form $\hat{\rho} W_0(\Phi, \Psi, t)$, where $\hat{\rho}$ is determined by entropy maximization. This introduces the free energy for given values of Φ and Ψ; W_0 is determined in the analysis of higher-order equations in ε as follows.

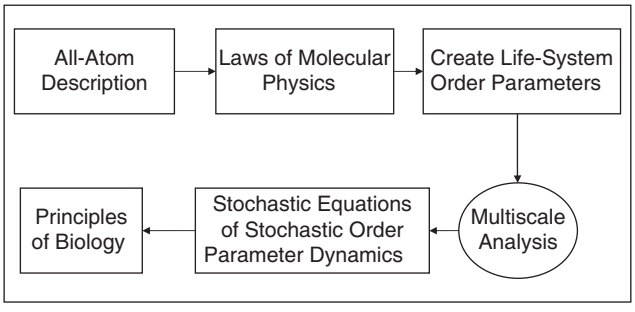


Fig. 3. The principles of microbiology are being derived from the laws of molecular physics via multiscale analysis and an all-atom starting description. Order parameters are created that translate atomic descriptions into biological variables that obey rigorous stochastic equations of self-assembly and transformation.

Before proceeding, we introduce the reduced probability density W for the order parameters. First, we derive an exact conservation equation for W. While this equation is not closed in W (i.e., depends on ρ and not just W), with the Taylor expansion for ρ we show that as $\varepsilon \to 0$ it is closed, $W \to W_0$, and the resulting dynamics occurs on the ε^{-2} timescale or longer.

Highlights of the final results are the following:

- All parameters in the final equations are calculated via formulas in terms of the interatomic force field that can (unlike for projection operator methods [3]) be evaluated via MD, constituting a calibration-free theory.
- The formalism breaks down when the factors appearing in the stochastic order parameter equations show anomalous (divergent) behavior, providing a self-consistency check. The remedy is to add more order parameters and repeat the analysis.
- When order parameter fields Ψ are included, the resulting equation determining W involves functional derivatives, and the Langevin equation for the stochastic dynamics of these order parameters involves a generalization of diffusion equations with thermal-average forces, nonlocal behaviors, and random force terms whose statistics are well characterized.
- The feedback of Figure 1 is incorporated in the theory, and the interaction of atomistic variables with discrete or field-order parameters is captured, all in a rigorous, self-consistent fashion.
- Our stochastic equations are generalizations of those introduced by Einstein and Smoluchowski to study Brownian motion. Although one of their objectives was to show that Brownian dynamics follows from the laws of physics, we complement this by developing a theory that captures the key role of Brownian motion in microbial systems.

From this methodology and results, we are deriving principles of microbiology.

Bioelectrics

Electrical forces between charges are strong and long range. Thus, the approach outlined earlier must be modified. Nonetheless, a multiscale perspective provides insights into bioelectric phenomena and a way to solve otherwise difficult equations.

Electrical effects in microbial systems are of two types. Electrostatics strongly influences bionanostructure and dynamics because of Coulomb interactions among charged groups on the structure and the counterion layers induced in the electrolyte from local variations in concentrations of mobile ions. Membrane potentials can be induced by active transport of ions or the restricted membrane permeability of some ions across the

Our objective is to use multiscale techniques to derive principles of microbiology from the laws of molecular physics.

membrane. In both bioelectric phenomena, one encounters multiscale effects. For example, the spatial variation of charge density and dielectric constant shows an angstrom scale variation, but the structures of interest, such as viruses, are of suprananometer size, counterion layers are on the nanometer scale in thickness, and bacteria are micrometers in size.

Electrostatic interactions among fixed charges on a nanostructure cannot be evaluated by considering Coulomb forces directly. This is due to the channeling of electrical fields induced by variations in the dielectric constant within the BNS, e.g., between an electrolyte and the interior of a globular protein. The Poisson–Boltzmann (PB) equation accounts for this field channeling and the shielding effect of the mobile ions in a host electrolyte. We have developed an efficient algorithm for solving the PB equation [5]. Our methodology follows from the recognition that the electrical potential depends on the position across the system in two distinct ways: 1) the angstrom scale variations reflecting the millions of charges fixed to the BNS and 2) the supranometer scale reflecting the overall variations in the field profile across the BNS. Multiple spatial scales play a key role in these systems. The thickness of the membrane, the thickness of counterion charge layers (the Debye length), and the size of the intracellular granules, organelles, or the whole cell are examples of these length scales. Using the ratio of the typical length associated with the distance between neighboring atoms (a few angstroms) to that of the overall size of the BNS (greater than a few nanometers), we derive rigorous coarse-grained equations for the overall field profile. This coarse-grained equation and the solution of a readily simulated linear equation for a response function yields an efficient multiscale PB solver. The solution technique preserves both angstrom and supranometer scale spatial variations in the electrical field. Its great computational efficiency over other PB solvers that do not take advantage of the multiscale nature of these systems will open up new opportunities in biosystem modeling.

Active transport across biological membranes and fixed charges in the medium create transmembrane electrical potentials. The strength of Coulomb forces creates equal and oppositely charged layers on opposite sides of the membrane. Thus, complete cell models must be electrometabolomic in character; the electrically driven transmembrane ion fluxes are coupled to the network of metabolic processes because of active transport.

Applications and Simulations

Whole-Virus Simulation in Energy and Health Sciences: The NanoX Software Package

Microbes in the soil, fuel tanks, petroleum reservoirs, and in engineered fermentation systems play a key role in energy generation, storage, loss, and transport [6]. For example, fuels stored in tanks of subsurface reservoirs can be degraded by a number of microbes. These microbes can be vulnerable to viral attack, presenting the possibility of designing vaccines against bacterial degradation of organic fuels. Viruses like poliovirus, human rhinovirus (HRV; human immunovirus), and human papilloma virus (HPV) are examples of nonenveloped systems, while human immunodeficiency virus (HIV) and dengue are enveloped viral human pathogens. In all these cases, we suggest that the whole-virus modeling can serve as a key element of a strategy for the computer-aided design of antiviral vaccines or drugs and for understanding the dynamics of natural and synthetic microbial colonies.

To this end, we have developed the NanoX all-atom multiscale simulation package (Figure 4). The virus we use to demonstrate our system is the well-characterized cowpea chlorotic mottle virus (CCMV) [7], [8]. The nonenveloped CCMV system undergoes a swelling transition induced by pH changes. Salinity, temperature, and Ca^{2+} concentration also regulate the transition. Order parameters for this and other BNSs were introduced to start our multiscale analysis as follows. Let $U_k(k = 1, 2, \ldots)$ be a complete set of functions of position $\vec{s}^0$ relative to the center of the simulation domain. We introduce vector-order parameters $\vec{\Phi}_k$ via a transformation, taking an atom i from its position $\vec{s}_i^0$ to $\vec{s}_i$:

$$\vec{s}_i = \sum_k \vec{\Phi}_k U_k\left(\vec{s}_i^0\right) + \vec{\sigma}_i. \tag{3}$$

As the k sum is finite (i.e., we seek only a minimal number of key order parameters), one must add a residual contribution $\vec{\sigma}_i$ to the more coherent contribution. We then determine $\vec{\Phi}_k$ to minimize the mass-weighted sum of the $\left|\vec{\sigma}_i\right|^2$, i.e., the set $\vec{\Phi}_k$ contains the maximum amount of information on the deformation of the BNS. With this and the orthogonality of the U_k, we find [9]

$$\vec{\Phi}_k = \sum_{i=1}^{N} \frac{m_i}{m^*} U_k\left(\vec{s}_i^0\right)\vec{s}_i, \tag{4}$$

where $\vec{s}_i$ and $\vec{s}_i^0$ are positions of atom i in a deformed and reference configuration of the virus. For example, $\vec{s}_i^0$ can be derived from an X-ray cryostructure, while $\vec{s}_i$ will be the structure as it evolves due to changes in pH. Also, m_i is the mass of atom i while m^* is the total mass of the atoms in the simulation domain.

The vector-order parameters have a center of mass (CM) character (for the particular basis functions U_k that vary smoothly over the simulation domain). $\vec{\Phi}_k$ changes slowly in time (as can be verified using Newton's equation). Thus, a viable starting point for our all-atom multiscale modeling of transitions in CCMV capsid using basis functions U_k in the form of

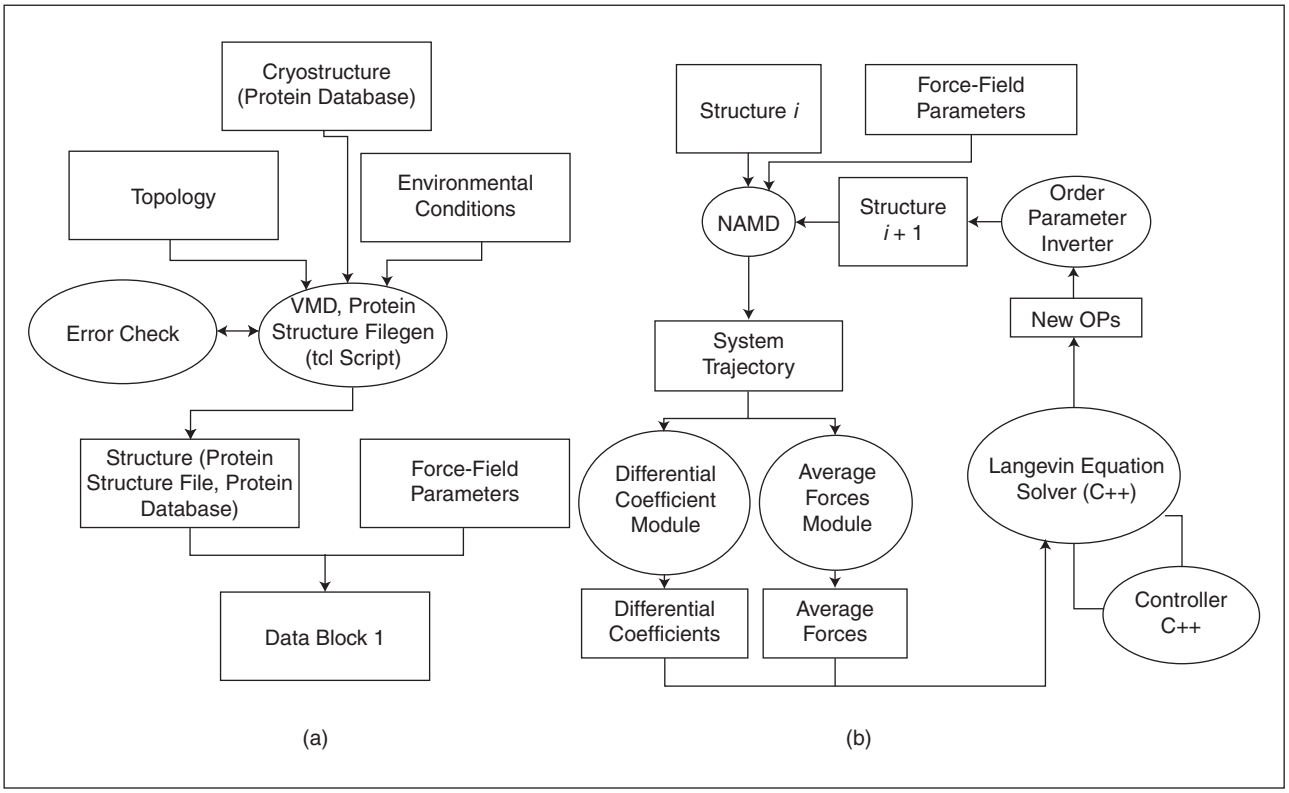


Fig. 4. (a) Schematic of the NanoX initialization algorithm. (b) Schematic of the NanoX main engine.

products of three Legendre polynomials, one for each (x, y, z) Cartesian axis. Using these order parameters and a computational algorithm based on the extrapolation of the system in time via the $\bar{\Phi}_k$ and the periodic short MD simulation [9], we evolve the capsid in time. As the number of order parameters used exceeds the number of hexamers and pentamers, this approach captures the swelling pathway proposed by Liu et al. [8] involving the displacement and rotation of capsomers.

With the structural order parameter $\bar{\Phi}_k$, an all-atom multiscale analysis (AMA) yields a Smoluchowski equation for $W(\Phi, t)$ of the form

$$\frac{\partial W}{\partial t} = \sum_{kk'} \frac{\partial}{\partial \vec{\Phi}_k} \bullet \left\{ \vec{\vec{D}}_{kk'} \left[\frac{\partial}{\partial \vec{\Phi}_{k'}} - \beta f_{k'} \right] W \right\}, \qquad (5)$$

where $\vec{f}_k = -\partial F / \partial \vec{\Phi}_k$ for Φ-dependent free energy F and $\vec{\vec{D}}_{kk'}$ is a tensor proportional to the integral in time of the correlation function

$$\left\langle \dot{\vec{\Phi}}_k (t)\, \dot{\vec{\Phi}}_{k'} (0) \right\rangle, \qquad (6)$$

with $\bullet$ implying a time derivative. A necessary condition for consistency of the AMA is that the correlation functions in (6) decay on a short timescale relative to that on which $\bar{\Phi}$ changes. Figure 5 shows this to be the case. The correlation time is on the order of that for single atom velocities [10] and is consistent with Figure 3. In Figure 6, we present a simulation of an order parameter capturing the overall size of CCMV (the same system for the correlation function in Figure 5). The correlation

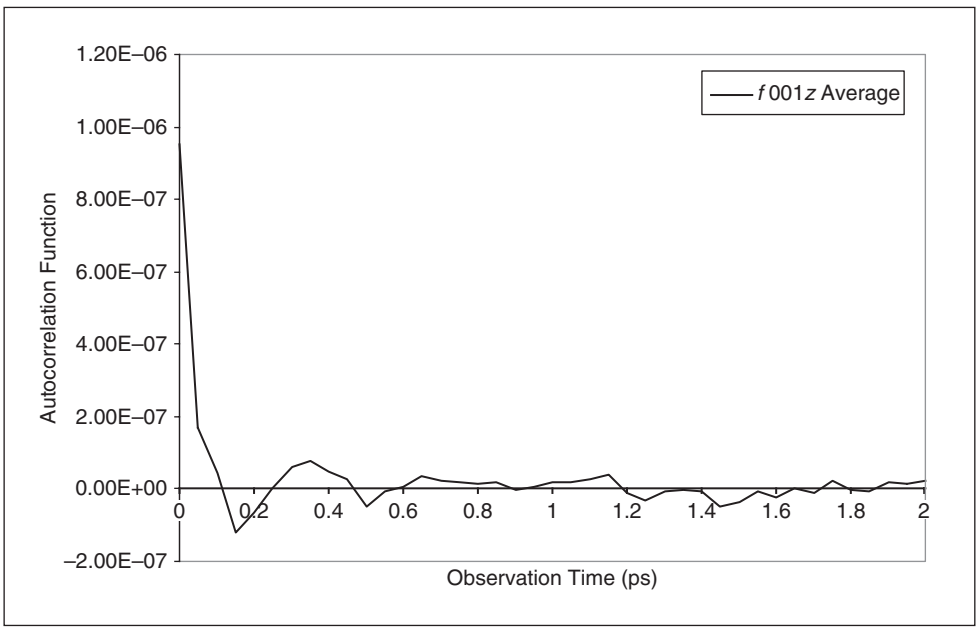


Fig. 5. Autocorrelation function of the 001z component of the thermal-average forces showing their short time character (see (3) for definition).

All-atom multiscale analysis addresses the challenge of predictive microbiological modeling for fundamental studies and for designing therapeutic strategies.

time is an order of magnitude or more shorter than the characteristic time for order parameter dynamics. Similarly, the thermal-average forces f_k drive the order parameters in a coherent manner. The diffusion matrix $\overline{\overline{D}}_{kk'}$ for various pairs kk' causes coupling such that f_k can create dynamics of Φ_k.

Nanocapsules for Delivery of Therapeutic Agents

Delivery of therapeutic small molecules, siRNA, or genes to diseased cells via nanocapsules holds a great promise for improving therapeutic efficacy and decreasing side effects. A well-designed nanocapsule can stabilize and isolate its payload until it reaches the target site; the payload must then be released (triggered by conditions at the target site), ensuring high drug concentration only at the target site. Considering the many, seemingly contradictory, criteria imposed on the nanocapsule design, we propose that a computer-aided strategy would accelerate progress.

The centerpiece of our computer-aided design strategy is the AMA-based NanoX simulator (sysbio.indiana.edu). 1) It does not require recalibration with each new application; 2) it accounts for the atomic scale detail necessary to evaluate interactions between a nanocapsule, the payload, host cell surface receptors, and other nanocapsules; and 3) it incorporates the interscale feedback of Figure 1. In the final regard, changes in local pH could induce a structural transition in the nanoscapsule, which could both induce its porosity and, via enhanced atomic-scale fluctuations, enhance the rate of transport across the nanocapsule.

In a preliminary study [11], this strategy was demonstrated using a set of order parameters specifying the state of the nanocapsule (i.e., position, orientation, and structure) and the payload (i.e., the CM position and the spatial extent of the cloud of payload molecules). The formulation yields the time course of release, the dynamical changes in nanocapsule permeability, and the effect of stochastic nanoscale dynamics. Thermal-average forces and diffusivities appearing in the stochastic equation are calculable via MD using AMA formulas. Alternatively, key parameters are identified that reduce the need for extensive calibration. A phenomenological equation relating our order parameters with the concentration profile was used to predict the drug release scenarios (Figure 7). Diffusion coefficients and thermal-average forces vary with biological conditions because of the changes in the free-energy landscape and mobility of major components due to pH, crowding, temperature, and other conditions.

Recently, an all-atom/continuum multiscaling (ACM) approach was developed that enabled the first self-consistent integration of atomistic and continuum theories [12]. Considering that the nanocapsule, the microenvironment, and the influence of stochastic forces on a nanocapsule or payload system are active, a simulation technique is needed that is more general than that used earlier. For example, the order parameters used in our virus studies assessed a long-lasting connectivity between the atoms (e.g., as in protein). However, the interdiffusional dynamics of a small molecule payload does not satisfy this criterion. To address this, field variables (e.g., the mass density profile for the drug) were introduced. This constitutes an uncountable number of order parameters, i.e., the field variables at each point in the system. Instead of the four discrete

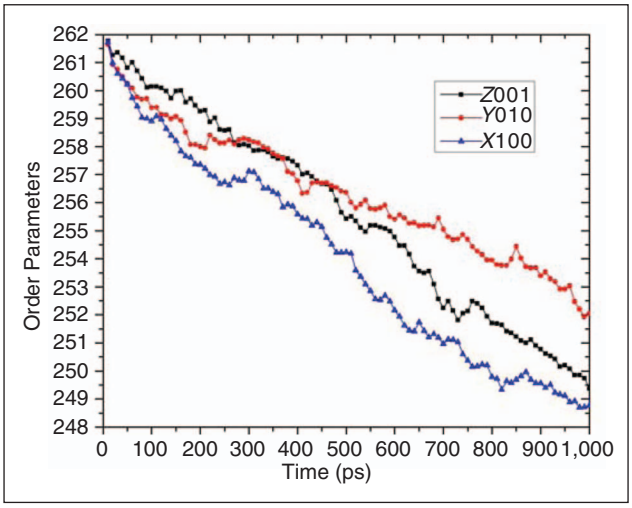


Fig. 6. Order parameters for the CCMV capsid showing their slow variation relative to the thermal-average force autocorrelation function (see Figure 5).

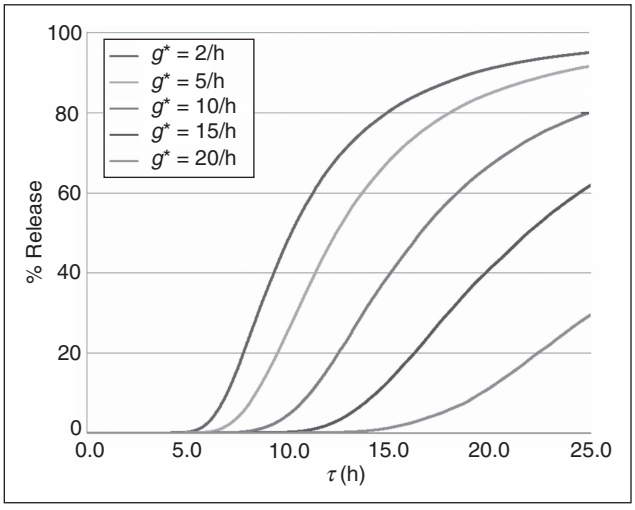


Fig. 7. Predicted release profile of doxorubicin from a liposome using different values of friction coefficients g^*. This shows that as the friction within the shell or barrier height increases, the release rate decreases.

order parameters introduced earlier [11], two field variables (mass density of the capsule material and that of the payload) were introduced [12]. This accounts for all the needed information on the position and overall structure of the nanocapsule and the dispersion of a cloud of payload molecules in a general way (e.g., without geometric restrictions). The result of ACM is a continuum generalization of the Smoluchowski equation for the stochastic dynamics of the field variables.

To illustrate ACM, consider the mass density field ψ. Our Smoluchowski equation takes the form

$$\frac{\partial W}{\partial t} = \varepsilon^2 \int \frac{d^3R}{v_c}\frac{d^3R'}{v_c}\frac{\delta}{\delta\psi(\vec{R})} \times \left\{ D(\vec{R},\vec{R}')\left(\frac{\delta}{\delta\psi(\vec{R}')} - \beta\bar{f}(\vec{R}')\right)\right\} W \qquad (7)$$

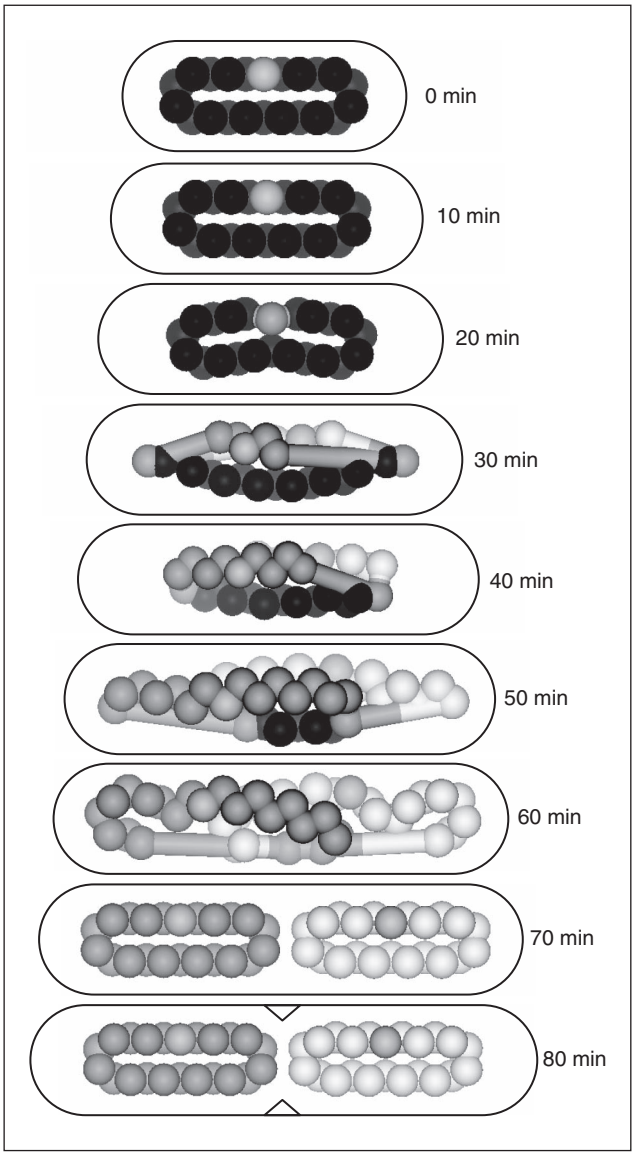


Fig. 8. Simulated configuration of *E. coli* chromosome structures. Pictured here are domains that contain the *ori* region, domains undergoing replication, domains of mother and domains of the two daughter chromosomes.

where $\vec{R}$ is a spatial point at which ψ is evaluated, v_c the minimal volume for which it is meaningful to speak of a field variable (contains a statistically significant number of molecules), f the thermal-average force, $D(\vec{R},\vec{R}')$ the diffusion coefficient, and W is the probability density as a functional of ψ. As mentioned earlier, $\delta/\delta\Psi(\vec{R})$ is a functional derivative and $f = -\delta F/\delta\psi(\vec{R})$ for free energy F. Through ACM, algorithms are provided for computing f and D so that, given the interatomic force field, we arrive at a calibration-free approach to the computer-aided design of nanocapsule delivery systems. This ACM formalism is also being explored for applications in virology and for bacteria as described later.

Bacterial Division: Self-Organization Pathways

The onset, maintenance, and dynamics of intrabacterial structure are the result of a highly orchestrated, micron-scale self-assembly dynamics. In our studies, we focused on the replication or division phenomena in *E. coli*. Our models integrate the formalism given in the first two subsections of the "Applications and Simulations" section. Although the mechanisms of eukaryotic daughter chromosome segregation and cell division have been elucidated to a certain extent, those for bacteria remain largely unknown. We developed a computational string model of *E. coli* chromosome segregation dynamics [13], [14]. The order parameters introduced were the CMs of postulated local condensed zones of the chromosomes of the original and daughter chromosomes. According to our AMA approach, these CMs satisfy Langevin equations. In a phenomenological approach, a novel thermal-average force field was postulated to account for stretching and bending, volume exclusion, and cell wall forces, all acting on the CMs of the condensed zones. The diffusion matrix, accounting for frictional effects, was assumed to be diagonal, i.e., each condensed zone experiences an average friction that is insensitive to the detailed configuration of the set of CMs or proximity to the cell wall. Langevin equations were simulated to model the motion of the CMs, and thereby, the chromosome structure changes. Chromosome mass was allowed to increase with replication. An extensive set of independent experimental data was used to calibrate the model. The mechanism of chromosome segregation was found to be the result of free-energy driven dynamics, i.e., the thermal-average forces were the gradient of the free energy with respect to the CMs. Predictions agree well with the observations of fluorescence-labeled chromosome loci movement in living cells. The results demonstrate the possibility of a mechanism of chromosome segregation that does not involve cytoskeletal guidance or advanced apparatus in *E. coli*. The model shows that DNA condensation into locally compacted domains is a requirement for successful chromosome segregation. Simulations imply that the shape-determining protein MreB may play a role in the segregation via modification of the cell wall force. An illustrative simulation is shown in Figure 8.

Population levels of various assemblies were recently posed as order parameters and a Smoluchowski equation, and Monte Carlo equivalent Langevin equations were derived for stochastic population dynamics [4]. When this theory is extended via a field variable approach as in the "Nanocapsules for Delivery of Therapeutic agents" subsection, one arrives at a stochastic continuum reaction-transport model. A phenomenological continuum model of this type, cast in terms of the concentrations of proteins and protein complexes in the cell

interior and on the inner surface of the membrane, was developed [14]. Using a reaction-transport model approach and a detailed model of active and passive biochemical processes (e.g., diffusions, dimerization, catalyzed removal or attachment of proteins to the intracellular side of the cell wall), we constructed a model of the self-organization of the division plane in *E. coli* based on the Min protein system. The simulation of Figure 9 is a depiction of simulated protein patterns for normal and abnormal *E. coli* morphologies. These phenomena were not correctly predicted by other models, wherein the self-organized patterns could only be predicted when the cell was much larger than normal. All simulations were carried out with our fully three-dimensional (3-D) finite element software Cell3D. The Cell3D simulator can be used to study cells of arbitrary size and geometry. The time average of the simulated normal and abnormal cell's patterns of Min protein distribution agrees well with the cell division plane location. As the cell shape and size are varied, the normal Min dynamics of an oscillatory pole-to-pole wave is transformed into a rotating or a bursting wave, wherein islands of surface-adsorbed MinD appear and disappear in an aperiodic spatiotemporal fashion.

The Cell3D simulator we developed accounts for Min protein adsorption or desorption at the intracellular-facing membrane surface and reaction and transport along this surface and in the interior of the cell. The surface [two-dimensional (2-D)] processes are coupled to those in the cell interior (3-D) through boundary conditions. The numerical approach overcomes technical difficulties with spherical coordinate-based cell simulators. A novel reaction network, involving the role of Min protein dimers and other observed complexes, makes Cell3D more complete and accurate than other models. All processes included were firmly based on observed reactions. Our results capture more observations than those presented by other groups (see Ref. [13]). The generality of stoichiometry and geometry of Cell3D makes it applicable to a broad range of cellular self-organization phenomena.

Bioelectricity

Structure, self-organization, and pattern of energy and mass flows in cells and bionanostructures are strongly affected by electrical forces and the associated multiscale phenomena they support. Our multiscale PB simulator PBms was demonstrated on a CCMV capsid. Considering that such a system requires a grid resolution of about 0.5 Å, a $(513)^3$ node grid was used. This makes a conventional PB simulation computationally intensive. PBms proved extremely efficient. It provides both atomic-scale and coarse-grained partial profiles, via our rigorous multiscale methodology [5]. Such potential profiles and associated electrostatic energy computations provide insights into the electrostatic effects in BNSs (e.g., pH and salinity [15]). A preliminary result on a viral capsid is shown in Figure 10. PBms takes 10 h to calculate the electrostatic potential isosurface on a single processor DELL Poweredge Xeon dual quad-core workstation. The alternative direction implicit (ADD) Poisson-Boltzmann (PB) solver by comparison takes 4 h on 16 processors on IBM SP Power4 machines [15].

An electrometabolomic model of *G. sulfurreducens* was developed, implemented as an extension of the Karyote cell simulator [16], and used to analyze the production of electrical current in a microbial fuel cell (Figures 11 and 12). The model integrates a number of factors including the current driven out of the cell via a redox complex, either along nanowires or via direct cell or electrode-surface interactions. Cell membrane potential-mediated transport of acetate H^+ and K^+ were accounted for. The existence of an intracellular substrate storage particle was postulated to explain the observed persistence of current after acetate concentration when the extracellular medium falls below detectable levels. The model appears to explain the characteristics of a *G. sulfurreducens* or acetate fuel cell and can thereby serve as the basis of a computer-aided fuel cell design strategy. Preliminary results suggest that the fuel cell performance can be enhanced by injecting acetate accompanied by a positive ion to which the microbial outer membrane is impermeable. For environmental applications, the model can be used for cases wherein oxidized minerals serve as the electron acceptor as well as to

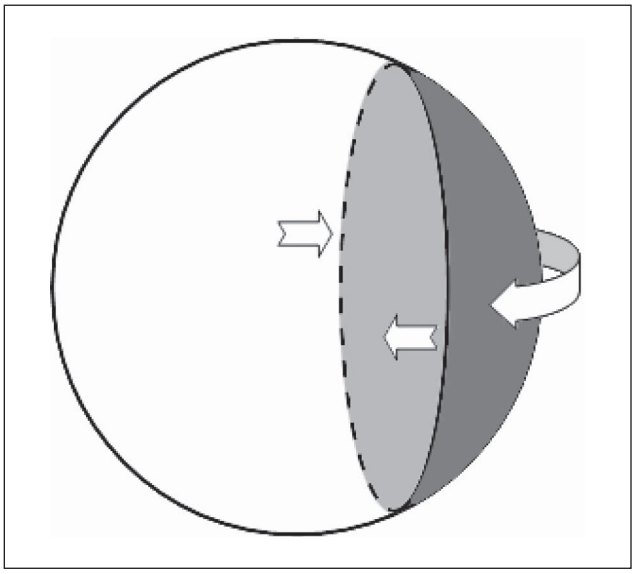


Fig. 9. Schematic spherical *E. coli* cell of radius 0.3 μm shows a rotating wave of MinD cloud. Because the cell size is too small to allow MinD reassembly at another site, MinD desorbs at the receding edge of the cloud and reabsorbs at the glowing edge, making the cloud appear to move across the spherical surface.

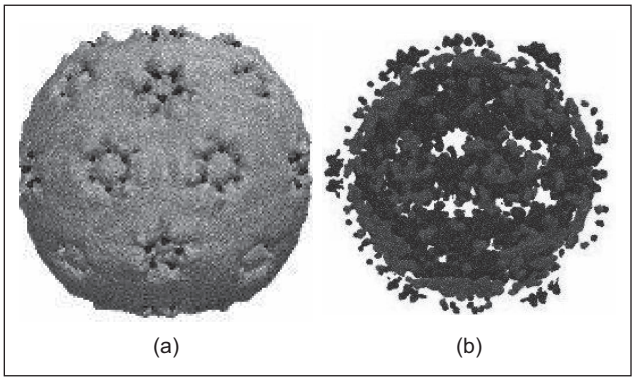


Fig. 10. $+5kT/e$ (black) $-5kT/e$ (gray) potential isosurfaces for the 432,240 atom CCMV capsid immersed in a 0.01 M 1:1 electrolyte (NaCl) computed via rigorous multiscaling. (a) External view. (b) Cut-away view.

study the dynamics when an organic substrate or mineral depletion leads to time-dependent effects.

Conclusions

Since the work of Einstein and Smoluchowski on Brownian motion, a deepened understanding of the role of stochastic forces in nanosystems has developed. As suggested in Figure 1, there is a feedback between the highly fluctuating (atomic) degrees of freedom and the more coherent, slowly varying ones (order parameters). We suggest that this conception provides a paradigm for understanding BNSs.

In our group, we have greatly extended this theme by a number of innovations in the analysis of the Liouville equation that enables the derivation of rigorous equations of the Smoluchowski or Fokker–Planck types that describe the dynamics of BNSs. Innovations include the following: 1) construction of sets of general order parameters that can automatically be increased in number to achieve self-consistency by eliminating memory effects, 2) avoidance of projection operators that introduce memory kernels that are difficult to evaluate, 3) an algorithm to derive equations for reduced probability densities that avoids integration over selected subsets of atomic variables and tedious bookkeeping to maintain the number of degrees of freedom, 4) use of an exact conservation law to gain an order in accuracy and to ensure conservation of probability in the final stochastic equation, 5) a scheme for integrating continuous field variables for disconnected subsystems and structural order parameters for connected substructures in one self-consistent theory via a novel hypothesis on the N-atom probability density, and 6) full accountability of the feedback across scales in space and time as in Figure 1.

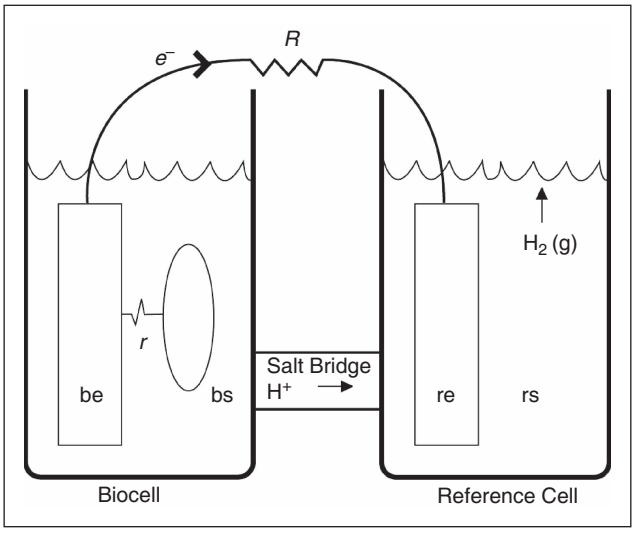


Fig. 11. Schematic microbial fuel cell showing the representative bacterium that sends electrons (e^-) down the nanowire of resistance r to the electrode across the application (of resistance R) and ultimately to the reference electrode.

Our objective is to use multiscale techniques to derive principles of microbiology from the laws of molecular physics. By multiscale techniques, we do not mean taking models at various spatiotemporal scales and adopting heuristic boundary conditions between them. This now popular technique is unfortunately fraught with inconsistencies and uncertainties. Rather, by rigorous, deductive multiscale techniques we derive a hierarchy of equations on increasingly longer scales. Considering the potential benefits of this approach (e.g., avoiding overcalibrated and inconsistent modeling), we believe that our approach could positively impact other multiscale modeling studies.

Although the multiscale character of these systems underlies computational difficulty, understanding it is also the key to addressing difficulties. The AMA we have developed accounts for the feedback between variables across scales in space and time. For example, atomic-scale fluctuations provide the entropic part of the free energy that drives the coherent dynamics of the large-scale features (order parameters) of the system. Conversely, the order parameters modify the probability distribution characterizing the range of atomic-scale fluctuations that are permitted. The conceptual flowchart of Figure 3 provides a road map for arriving at equations for the stochastic dynamics of the order parameters. Our equations provide a computationally feasible approach to simulating microbiological phenomena. Because all factors in these equations are related to the interatomic force field via rigorous statistical mechanical expressions, our AMA addresses the challenge of predictive microbiological modeling for fundamental studies and for designing therapeutic strategies. We are developing a

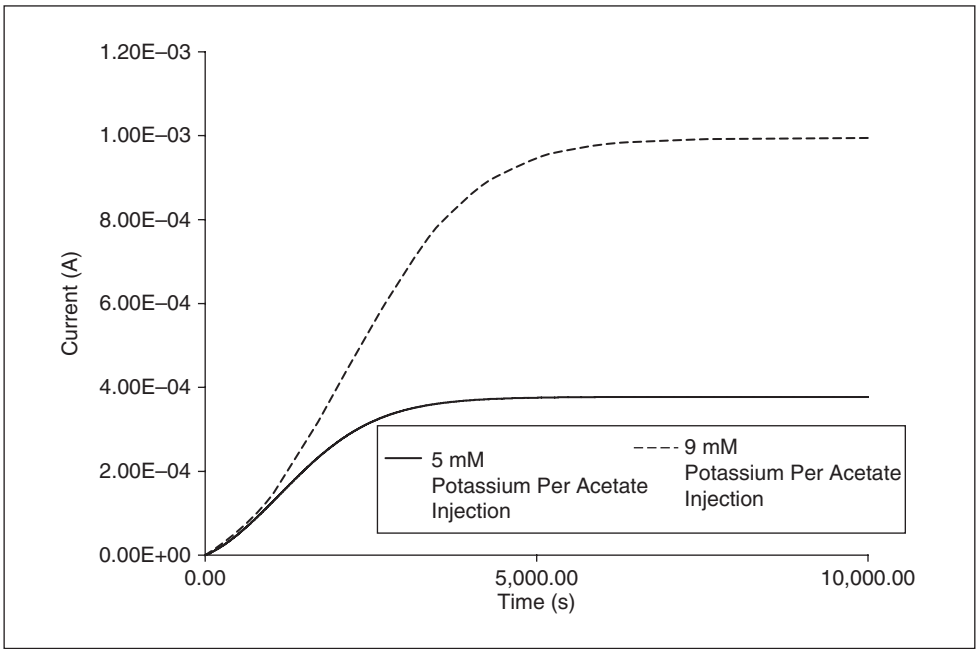


Fig. 12. Response of the microbial fuel cell-generated current at 5 and 9 mM injections of potassium per acetate.

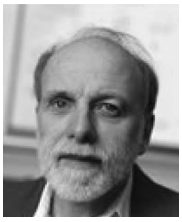
multiscale software for the design of antiviral drugs and vaccines. This software will enable us to predict strains of known viruses with pandemic potential, enabling a preemptive strategy where potential threats to global health could be addressed via an ongoing computational effort.

To make our advances accessible to other researchers, we make the following microbial systems modeling software available through our Web portal (sysbio.indiana.edu):

- Karyote
- Cell 3D
- PBms
- CellX
- NanoX.MD/OPX
- NanoX.multiscale.

Acknowledgments

This project at the Center of Cell and Virus Theory was supported by the National Institutes of Health and the U.S. Department of Energy.



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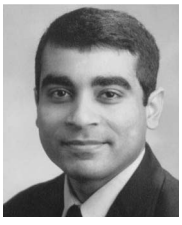

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References

[1] D. Harries, S. May, W. M. Gelbart, and A. Ben-Shaul, "Structure, stability and thermodynamics of lamellar DNA-lipid complexes," *Biophys. J.*, vol. 75, p. 159, 1998.

[2] A. Arkhipov, P. L. Freddolino, and K. Schulten, "Stability and dynamics of virus capsids described by coarse-grained modeling," *Structure*, vol. 14, pp. 1767–1777, 2006.

[3] R. Zwanzig, *Nonequilibrium Statistical Mechanics*. New York: Oxford Univ. Press, 2001.

[4] S. Pankavich and P. Ortoleva, "Self-assembly of nanocomponents into composite structures: Multi-scale derivation of stochastic chemical kinetic models," submitted for publication.

[5] A. Roy and P. J. Ortoleva, "Multiscale Poisson-Boltzmann approach to nanosystem electrostatics," unpublished.

[6] M. Fontus and P. J. Ortoleva, "An electrometabolomic model of the *G. sulfurreducens* microbial fuel cell," submitted for publication.

[7] J. A. Speir, et al., "Structures of the native and swollen forms of cowpea chlorotic mottle virus determined by X-ray crystallography and cryoelectron microscopy," *Structure*, vol. 3, no. 1, pp. 63–78, 1995.

[8] H. J. Liu, et al., "Pseudo-atomic models of swollen CCMV from cryo-electron microscopy data," *J. Struct. Biol.*, vol. 142, no. 3, pp. 356–363, 2003.

[9] Y. Miao and P. J. Ortoleva, "Molecular dynamics/order parameter extrapolation (MD/OPX) for bionanosystem simulations," *J. Comput. Chem.*, to be published.

[10] D. M. Heyes, "Physical properties of liquid water by molecular dynamics simulations," *J. Chem. Soc. Faraday Trans.*, vol. 90, no. 20, pp. 3039–3049, 1994.

[11] Z. Shreif and P. Ortoleva, "Computer-aided design of nanocapsules for therapeutic delivery," *Comp. Math. Methods Med.*, to be published.

[12] Z. Shreif and P. Ortoleva, "All-atom/continuum multiscale theory: Application to nanocapsule therapeutic delivery," submitted for publication.

[13] J. Fan, K. Tuncay, and P. J. Ortoleva, "Chromosome segregation in *E. coli* division: A free energy-driven string model," *J. Comp. Biol. Chem.*, vol. 31, pp. 257–264, 2007.

[14] J. Fan, "Simulated chrosome segregation and min protein dynamics in prokaryotic cell division," Ph.D. thesis, 2007.

[15] A. Sayyed-Ahmad, Y. Miao, and P. J. Ortoleva, "Poisson-Boltzmann theory for bionanosystems," *Commun. Comput. Phys.*, vol. 3, no. 5, pp. 1100–1116, 2007.

[16] P. Ortoleva, E. Berry, Y. Brun, J. Fan, M. Fontus, K. Hubbard, K. Jaqaman, L. Jarymowycz, A. Navid, A. Sayyed-Ahmad, Z. Shreif, F. Stanley, K. Tuncay, E. Weitzke, and L. C. Wu, "The karyote physico-chemical genomic, proteomic, metabolic cell modeling system," *OMICS*, vol. 7, no. 3, pp. 269–283, 2003.