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Complex Biological and Bio-Inspired Systems

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Overview

The understanding and characterization of the fundamental processes of the function of biological systems underpins many of the important challenges facing American society, from the pathology of infectious disease and the efficacy of vaccines, to the development of materials that mimic biological functionality and deliver exceptional and novel structural and dynamic properties. These problems are fundamentally complex, involving many interacting components and poorly understood bio-chemical kinetics. We use the basic science of statistical physics, kinetic theory, cellular bio-chemistry, soft-matter physics, and information science to develop cell level models and explore the use of bio-mimetic materials. This project seeks to determine how cell level processes, such as response to mechanical stresses, chemical constituents and related gradients, and other cell signaling mechanisms, integrate and combine to create a functioning organism. The research focuses on the basic physical processes that take place at different levels of the biological organism: the basic role of molecular and chemical interactions are investigated, the dynamics of the DNA-molecule and its phylogenetic role are examined and the regulatory networks of complex biochemical processes are modeled. These efforts may lead to early warning algorithms of pathogen outbreaks, new bio-sensors to detect hazards from pathomic viruses to chemical contaminants. Other potential applications include the development of efficient bio-fuel alternative-energy processes and the exploration of novel materials for energy usages. Finally, we use the notion of "coarse-graining," which is a method for averaging over less important degrees of freedom to develop computational models to predict cell function and systems-level response to disease, chemical stress, or biological pathomic agents.

This project supports Energy Security, Threat Reduction, and the missions of the DOE Office of Science through its efforts to accurately model biological systems at the molecular and cellular level. The project's impact encompasses applications to biofuels, to novel sensors and to materials with broad use for energy or threat reduction.
The broad, interdisciplinary approach of CNLS offers the unparalleled strength of combining science backgrounds and expertise – a unique and important asset in attacking the complex science of biological organisms. This approach also allows cross-fertilization, with concepts and techniques transferring across field boundaries.

Introduction

Although biological organisms remain systems of daunting complexity, powerful new techniques are making inroads in unraveling the underlying structures and mechanisms of life. Novel probes such as optical tweezers, single molecule tracking and nanotechnology methods yield data of unprecedented detail. Large, world-wide data-sets on DNA sequences can be shared, stored and analyzed using computers of unparalleled power. More than ever before, the increased quantitative and detailed nature of the measured data is making biological studies amenable to the powerful techniques and methods of physics and statistics. The advances in nanotechnology and material science, moreover, have resulted in new tools such as nano-particles that attach themselves to large molecules to study transport and functionality and biomimetic materials. The CNLS-research in this area focuses on the use of physical concepts, statistical and numerical techniques and nonlinear physics to reveal the nature of biological processes. Insights gained from these studies are at the forefront of epidemiology, bio-informatics and bio-sensing and of direct interest to threat reduction. Understanding the biochemistry and regulation of biological systems promises to help with energy-harvesting and can contribute to energy-security.

CNLS postdoctoral research in theses areas can be divided according to the scale of the bio-components that are being investigated using chemical, physical, statistical and numerical methods. (A) At the most basic level, techniques of non-equilibrium and statistical physics are employed to study the role of inter-atomic and molecular interactions. (B) At the level of the DNA-molecule and its phylogenetic role, the techniques of sequencing and DNA-data analysis and the modeling of non-linear DNA dynamics are revealing fundamental aspects of the role of DNA biology. (C) At the cell-level and in the bio-informatics realm, modeling and stochastic descriptions are uncovering intricate connections in the regulatory mechanisms that govern the life and the function of cells.

A. Inter-atomic and molecular interactions in biology

CNLS researchers are using the tools of statistical physics to understand the stability and dynamics of cellulose structures. Cellulose is a major component of biomass. It is universally important in all forms of usable biomass, from plants and algae to coral reefs. As an assembly of polymers of monosaccharide, it can be converted to glucose, see Fig. 1, and eventually to biofuel ethanol, which is a hotly pursued alternative, renewable energy resource. The practical problem we face today with such an approach is the high stability of the assembly, and thus inefficient way to depolymerize. In vivo, only when interacted with a very specific class of enzymes (a set of proteins called cellulase), the cellulose can be turned into a solution of monomers. This year, we constructed a model that details the essential physical interactions (both between polymer chains and inside each polymers) using hydrogen bond network [Shen & Gnanakaran, 2009]. This model

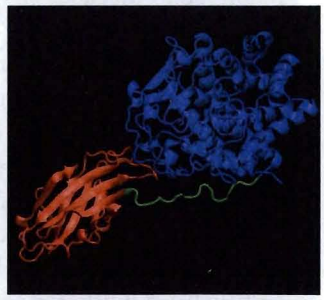


Fig. 1 Schematic of enzymatic degradation

successfully describes the effects of temperature on the rupture of hydrogen bonds and the disassembly of crystalline cellulose I-beta. The predictions made by this model are consistent with the trends observed in experiments and MD simulations. Many new insights obtained from this model will be also tested in future experiments. Other different crystalline phases of the cellulose will be computed and compared in the near future. Disordered phases that can be created in the assembly process will be computed as well.

Besides attacking the problem from the coarse-grained model with a partition-function based theory, we also study the assembly at the all-atom simulation level [Shen and Hamelberg, 2008]. Multiple simulations at different temperatures and setups conducted give us valuable information on the flexibility of the conformations of the cellulose fibers and other thermodynamic and mechanical properties. These calculations will be used to explain many important aspects of the assembly, especially those that are relevant to enzyme cellulose interaction with cellulose.

We have also focused on two related problems, one of which explores the role of interactions in creating selective transport through nano-channels. The other project, not funded under this proposal, deals with a very different biological systems –both are united by a common theme: the application of the methods of non-equilibrium statistical mechanics and the theory of stochastic processes to real biological problems. In this respect, both projects have been cross-pollinating.

Functioning of living cells requires selective transport of molecules into and out of the cell nucleus, see Fig. 2. This transport is also important in the replication of certain viruses, such as influenza, that replicate in the nucleus. Transport occurs through channels that are able to selectively move only certain molecular species while effectively filtering out others, even very similar ones. Moreover, such channels can selectively transport their specific molecules in the presence of vast amounts of non-specific competitors. In many biological channels, efficient and selective transport occurs without direct input of metabolic energy and without transitions from an 'open' to a 'closed' state during the transport event. Examples include selective permeability of porins and transport through the nuclear pore complex in eukaryotes. Mechanisms of selectivity operational in such channels hold a great potential for applications in nano-technology and nano-medicine, which recently has lead to design artificial selective nano-channels, which mimic the selective biological channels and are built on the principles operational in those.

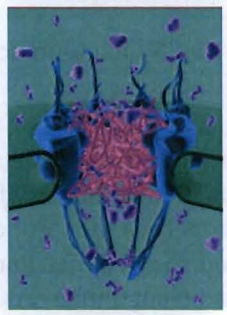


Fig. 2 Nuclear pore complex

Precise mechanisms and conditions of selective transport through such nano-channels are still unknown. CNLS researchers have been developing theoretical models to explain the mechanisms of selectivity of transport through nano-channels, which contains only two essential ingredients: -i) transient trapping of the cargoes inside the channel (e.g., owing to transient binding to moieties inside the channel), and ii) competition between the

transported molecules for the limited space inside the channel. Some of this work [Jovanovic-Talisman et al, 2009; Zilman et al, 2009] has been done in a close collaboration with the experimental colleagues at Rockefeller University who have been working on implementation of the concepts in a real nano-molecular filter. The theory [Zilman, 2008] provides a mechanism for selectivity based on the differences in the kinetics of transport through the channel between different molecules. The theory explains how the specific molecules are able to efficiently filter out the non-specific competitors – and proposes a mechanism for sharp molecular discrimination. The theory also provides a blueprint for future creation of ever more selective artificial nano-channels. The theoretical predictions explain previous experimental results and have been verified in ongoing experiments at the Rockefeller University.

Single molecule tracking experiments, widely used in biology, may reveal more information about specific biological systems. CNLS researchers analyzed a set of data that recorded the trajectories, Fig. 3, of a quantum dot attached to receptor on the surface of a living cell. The analysis of the trajectories shows that the motion is not a free diffusion of the quantum dot. In order to understand the mechanism that underpins the receptor motion, we designed a minimal model to explain the data. We derived analytical expressions for the probability of the trajectories within this model. Our next goal is to obtain model parameters that maximize the probability of the observed trajectories. We are currently writing a computational code to determine the model parameters and hope to get more data in the future to test the model with better statistics. We will also consider other models and compare the fits to these models in order to decide which model most successfully describes the quantum dot motion. To understand under which conditions molecular interactions may affect the diffusion properties of a probe, we considered a theory of general interactive diffusive particles, and showed than anomalous diffusion arises at arbitrarily weak interaction strengths [Bel and Nemenman, 2009].

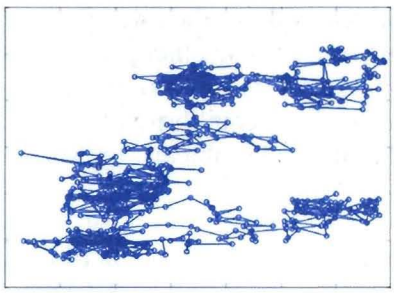


Fig. 3 Quantum dot particle tracks

In related work on single molecules, we consider the emission spectroscopy of a single molecule [Bel and Brown, 2009]. Whereas most previous works considered only the statistics of the total number of photons emitted by the excited molecule, our work considers the statistics of the number of photons emitted with a given frequency, as well as correlations between photons at different frequencies. We are also calculating the correlation between photons from different atomic transitions.

B. DNA-level dynamics and epidemiology

CNLS researchers are working on the nonlinear description of DNA dynamics and its connection with biological function, i.e., transcription, mutation and genetic diseases. This work takes the phenomenological approach incorporated in the Peyrard-Bishop-

Dauxois (PBD) model of DNA molecules, see schematic illustration in Fig. 4. We are using the model to describe the connection among the nonlinear dynamics of double-stranded DNA, experimental findings and specific DNA functions [Alexandrov et al, 2009]. At the same time, we are improving the model to better characterize these complex dynamics. In particular, we have shown how thermally induced localized openings (or bubbles) of the double strand are important for interpreting dynamic force spectroscopy data. There is a correlation between a sequence-dependent tendency for pre-melting bubble formation and transcription initiation and other regulatory effects in viral DNA. In addition, we are exploring the possibility of a connection between DNA dynamics and the ability of repair proteins to recognize ultraviolet radiation damage sites.

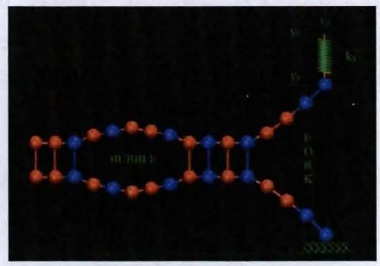


Fig. 4 PBD model of a dynamic force experiment.

Los Alamos has one of the worlds' strongest theoretical efforts in the immunology of HIV. CNLS researchers are helping develop and optimize HIV sequence analysis methods to ultimately improve the design of vaccines. HIV is the most variable organism known to modern biology, in no small part why no effective vaccine has been created. Our inability to develop a vaccine is influenced by a lack of knowledge about the viral variation during the disease progression, as well as during transmission. To investigate this, CNLS researchers have created computational methods to find unique features of the virus during early infection, which may help design vaccines [Kulkarni et al, 2009; Zhang, 2009a]. On the pandemic level, we have investigated almost 10,000 HIV sequences, obtained from three epidemiologically distinct settings, and have demonstrated that old and new HIV sequences are co-circulating, and that modern recombinant strains are moving toward more complex genomic compositions [Zhang et al, 2009b]. This situation poses a challenge to any attempted vaccine design. Accurate classification of the diverse worldwide HIV epidemic, including identification of complex recombinant forms, is also important from an epidemiological perspective [Bulla et al, 2009; Schultz et al, 2009], where the different forms are associated in a dynamic way with different risk groups and geographical locales. Furthermore, CNLS researchers are developing phylo-dynamic methods, based on the DNA substitution rate of HIV on the population level, that can reveal how fast it is spreading [Maljkovic Berry et al, 2009; Ha et al, 2009]. This may become an important epidemiological tool that can signal when outbreaks are starting, and both direct and inform vaccine development and other prevention efforts.

At the molecular level, messenger RNA transcribes DNA to form proteins through the reading of the RNA by the ribosome. In addition, however, a large fraction of RNA does not code for proteins and its biological function is far from certain. CNLS researchers are collaborating with researchers at the Scripps Research Institute on human non-coding RNA that accounts for 98% of the human genome. We created an automated procedure to screen cis-antisense pairs from non-coding RNAs with particular emphasis on short

sequences [Zhang et al, 2009c]. We also were able to screen for microRNAs that are able to bind with high affinity to sense-antisense pairs in the human genome. The results of this work are currently be prepared for publication.

C. Bio-informatics and modeling complex bio-chemical networks

During the past year, CNLS researchers collaborated with scientists at the Chemical & Biomolecular Engineering Department, NCSU on modeling reaction-diffusion interactions at platelet-derived growth factor (PDGF) receptors, which are responsible for regulating cell growth and division. This is related to but separate from work funded under different auspices on development of rule-based models for simulating membrane-proximal reactions in cells.

Intracellular signaling pathways often depend on enzymes that are recruited by plasma membrane receptors and act upon membrane-associated substrates. Thus, the membrane acts as a physical platform for interactions taking place at the early stages of receptor-mediated signaling, bringing enzymes closer to their substrates. We have developed a quantitative model that expands on the role of diffusion-controlled kinetics — a two-dimensional Brownian dynamics kinetic model, see Fig. 5. The model introduces "spatial coupling" whereby simultaneous recruitment of different enzymes to the same receptor facilitates crosstalk between different signaling pathways [Monine & Haugh. 2008a]. We analyzed the specific case of phosphoinositide 3-kinase (PI3K), which is localized as a result of cooperative interactions between receptors and active Ras (membrane-associated protein). The assembly of receptor/PI3K/Ras complexes is facilitated by the local action of a guanine exchange factor (GEF) bound to the same receptor. Spatial coupling between GEF and PI3K relies on Ras being first released by a receptor-bound GEF and then captured by a PI3K molecule associated with the same receptor. We evaluated the probabilities of short- and long-range interactions to characterize spatial coupling. We have also built a macroscopic model of dermal wound healing [Monine & Haugh, 2008b], which accounts for the PDGF gradient sensing mechanism in tissue cells (fibroblasts). Our hybrid modeling approach treats fibroblasts as discrete objects endowed with heterogeneous properties, namely expression levels of PDGF receptors, PI3K and other enzymes. Analysis of the model suggests that cell-to-cell variability results in a significantly higher rate of wound invasion as compared with a case of non-distinct cells, in a manner that depends on how individual cell properties are sampled or inherited upon cell division.

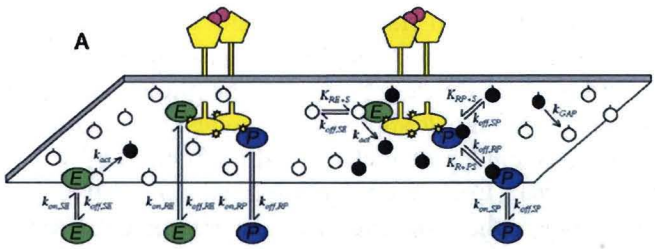


Fig. 5 Biochemical binding/reaction network

In other work, we have developed simulation methods and tools for modeling biochemical systems and we have applied these methods and tools to study various aggregation phenomena that play a role in cell signaling (Yang et al., 2008; Colvin et al., in press; Nag et al., in press; Monine et al., submitted).

CNLS researchers have explored a complementary approach, namely coarse-grained phenomenological models, and have produced the most comprehensive, and yet one of the simplest models of *lac* regulation in *E. coli*, which, for the first time, makes predictions about the domain of the bistability of this well-studied regulatory circuit [Dreisigmeyer et al., 2008].

There is considerable effort among CNLS researchers and affiliates on describing cell function through a thorough understanding of biochemical processes in the cell, such as understanding statistical properties to completion of complex reaction steps [Munsky, Bel, and Nemenman, in prep.] This work has helped produce a numerical algorithm that speeds up simulations of stochastic biochemical processes by several orders of magnitude (in time) without loss of information about the stochastic characteristics of the chemical reactions [Sinitsyn, Hengartner, and Nemenman, 2009]. This work is based on an earlier development of a powerful set of tools of adiabatic quantum mechanics in the context of biochemical kinetics, which led to the discovery of a totally unexpected geometric phase phenomenon in classical stochastic systems [Sinitsyn and Nemenman, 2007; Sinitsyn and Nemenman, 2007a] and a series of algorithms for reverse-engineering enzymatic reaction kinetic diagrams using fluctuations data [de Ronde et al., 2009].

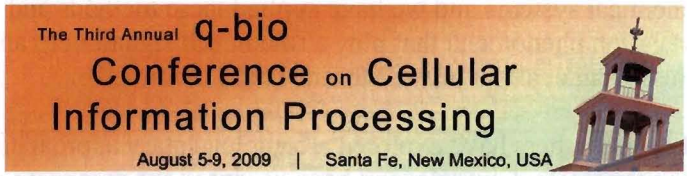
In related work, CNLS scientists collaborated with a CNLS visitor to discover the pumping-restriction theorem, which is an exact non-perturbative result that imposes strong restrictions on the response of a mesoscopic system to periodic perturbations [Chernyak & Sinitsyn. 2008]. The result will find applications in the reconstructions of biochemical network topologies, and it complements nicely another direction pursued by CNLS summer students with a similar goal, and yet performed using very different tools, namely statistics and information theory [Nemenman et al., 2007].

Finally, a major CNLS effort in analysis of biochemical regulatory networks is in understanding the constraints that the topological, molecular number, temporal responsiveness, and other constraints put on the quality of information processing in these networks. CNLS summer students have discovered that a generic biochemical network possesses a surprisingly high fidelity, is highly adaptable, and yet robust to most of its parameter changes [Ziv et al., 2007; Mugler et al., 2008; Mugler et al., 2009].

Related Activities

In 2008, CNLS co-sponsored 2 conferences on quantitative biology that significantly advanced the overall state of research at LANL and helped build national and international collaborations in this emerging field. Building on the 1st Annual q-bio Conference in 2007 [Edwards et al, 2007], the 2nd q-bio Conference was a huge success,

attracting outstanding researchers from around the world, including talks from 5 NAS members. A special issue in *IET Systems Biology* dedicated to work presented at the 1st q-bio Conference appeared in September 2008 [Nemenman et al, 2008]. Special issues dedicated to work presented at the 2nd q-bio conference will appear in *Molecular Systems Biology* and *IET Systems Biology* in 2009. The q-bio Conference is dedicated to advancing predictive modeling of cellular regulatory systems. The emphasis is on modeling and quantitative experimentation for understanding and predicting the behaviors of particular regulatory systems, phenomena that manifest themselves in many biological systems, and/or general principles of cellular information processing. The 3rd q-bio Conference is scheduled for August 2009 and has attracted external support from the NIH.



A satellite conference to the 2008 q-bio Conference was on the subject of the nuclear pore complex (NPC). The workshop helped foster the interaction between experimental and theoretical approaches aimed for relating NPC structure to its function, and furthered the understanding of the mechanisms of selectivity of transport through the NPC and related biologically-inspired artificial molecular sorting nano-devices.

In 2007 and, to a lesser extent in 2008, CNLS has also participated in organization of the q-bio Summer School in Los Alamos, preceding the conference. The event attracts students with a natural sciences background interested in modeling biological systems. Over three weeks of the conference duration, the students have been able to learn enough and to establish strong enough ties with LANL staff to produce multiple publications as a result of the school activities [Dreysigmeyer et al., 2008; Mugler et al., 2008; Mugler et al., 2009; de Ronde et al., 2009].

In September 2008, CNLS initiated a weekly seminar series and a monthly series of public lectures dedicated to quantitative biology. 19 external speakers and 2 internal speakers have participated in the seminar series so far. Public lectures typically take place on the third Tuesday of every month in Santa Fe at the Santa Fe Complex. The lectures have been widely covered by local media, with lecturers typically being interviewed on local radio stations.

Finally, the CNLS Annual Conference entitled "Energy for the 21st Century" to be held May 18-22, 2009 will include a substantial section on the science and technology of bio-fuels. The overall purpose of the conference is to provide an open forum for active interactions between academic, government and industrial researchers from different subfields to debate the issues of our energy future. The subjects of the discussion include, but are not limited to: science and technology of renewable (solar, wind, tidal, biomass, geo-thermal) and non-renewable (e.g., fossil, nuclear) energy sources, energy storage and transmission, as well as global economic, climate and geo-political issues.

Summary

By exploring biological processes from the molecular level up to the full complexity of the cellular level, we are beginning to obtain a quantitative picture of the complexity of biological function. By building accurate models of how molecular processes interact within a cell to create cell specificity and function, we can begin to manipulate those functions for new applications. A specific example is designing an efficient biological biomass conversion process that could help address energy sustainability issues. Other implications of our work include better predictability of the spread of contagious disease and the design of effective vaccines for such diseases. Using these biological systems and their models as a basis, we can design new materials that mimic the function of membranes, vesicles, and muscle. As we move forward in the second part of this proposal funding cycle, we hope to utilize progress made earlier to advance these applications.

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