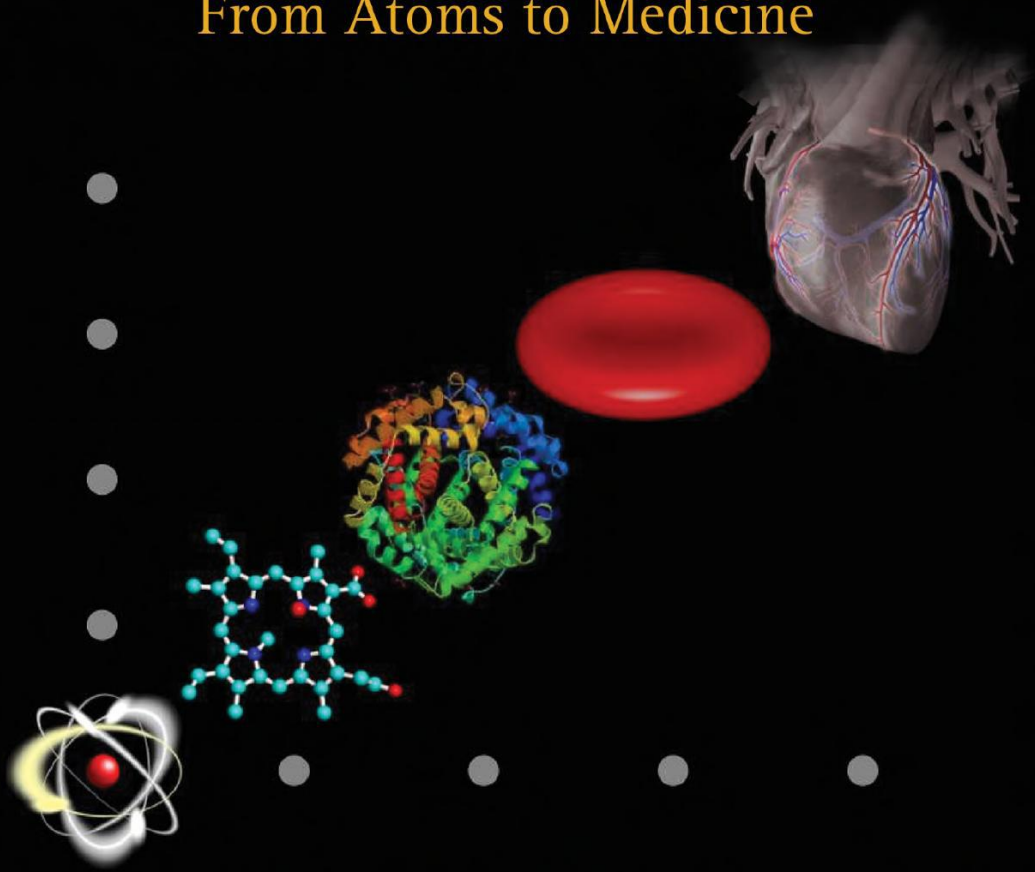
PHYSICAL BIOLOGY

From Atoms to Medicine



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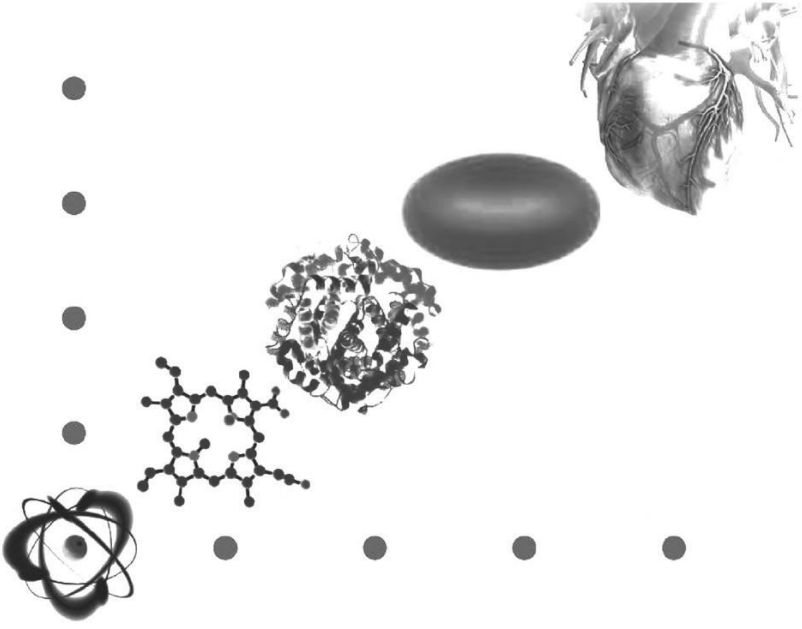

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Ahmed H. Zewail
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Contents

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Prologue

Some historians of science are inclined to classify fields and their impact with a broad brush — we are told that chemistry was the science of the 19th century, physics the science of the 20th, and that biology is the science of the 21st century. Other historians, such as the renowned Thomas Kuhn, discussed the concept of a paradigm shift and defined a separation for the impact of concepts and tools. As a practicing scientist I, in fact, see a simpler picture with less of a chasm in the progress of science. Biology without concepts and tools from physics, chemistry, and now engineering, would be severely limited. Tools are as important as concepts and, at the end, the two amalgamate to change the way we observe and the way we think.

We may recall the impact on biology of tools such as X-ray diffraction, electron microscopy, and NMR. Related and equally significant is the central role of concepts, such as chemical bonding, dynamics of transition states, and molecular recognition, in elucidating mechanisms of biological activity. From biology, the tools of RNAi, PCR, site-directed mutagenesis, and gene knockout are opening up new avenues of chemical syntheses and physical studies. Similarly, concepts of complementarity, self-assembly, and collective interactions (coherence) of molecular machines are introducing to physics and chemistry challenges and opportunities in the realm of emergence and complexity.

This volume brings about the confluence of concepts and tools and that of different disciplines to address significant problems of our time in physical biology and adjacent disciplines. Specifically, the book is structured to provide a broad perspective on the current state-of-the-art methods and concepts at the heart of chemical and biological behavior, covering the topics of visualization; theory and computation for complexity; macromolecular function, protein folding and misfolding; molecular recognition; and

systems integration from cells to consciousness. The scope of tools is wide-ranging, spanning imaging, crystallography, microfluidics, single-molecule spectroscopy, and synthetic probe targeting, either molecular or by metallic particles. The perspectives are made by world leaders of physics, chemistry, and biology, and they define potential new frontiers at the interface of these disciplines, including physical, systems, and synthetic biology.

The book, which is the product of the 2007 Welch Conference on Chemical Research, is not a proceeding of reports and references. The articles are overviews, rather than reviews. They provide a panoramic view across areas of progress made, from revolutionary tools for the determination of extended structures to perspectives on 21st century biology and medicine. It was pleasing to welcome an audience of nearly a thousand people, surely reflecting the excitement about the contributions and the contributors. Personally, I wish to thank all the contributors who accepted our invitation and made this volume possible. Out of the twenty scientists invited to the conference, only John Walker was unable to travel at the time of the gathering in Houston on October 22, 2007. Two colleagues, Susan Lindquist of MIT and Julie Theriot of Stanford University, were invited but could not participate.

The Houston event would not have been possible without the generous support of The Welch Foundation and its leaders. The contribution to the organization made by the president of the foundation, Mr. Norbert Dittrich, and his staff, especially Ms. Carla Atmar, is greatly appreciated. Over the years, the Welch conferences have benefited from the wisdom of members of the Scientific Advisory Board, chaired formerly by the late Norman Hackerman and now by James L. Kinsey. As a member, I have enjoyed and benefited from the wide-ranging and stimulating discussions, which in one way or another have influenced the outcome of this endeavor. During this event we celebrated the achievements of two distinguished colleagues, Noel Hush and William Miller, who both received the 2007 Welch Award for their contributions to theoretical chemistry — their addresses are happily included in this volume.

(*From left*)
Norbert Dittrich,
Ahmed H. Zewail
and James L. Kinsey.

The timely completion of this book demanded careful orchestration among all contributors while the editor was in Pasadena or on travel. Essential to this coordination was the support of my assistants at Caltech, De Ann Lewis and Maggie Sabanpan. De Ann's special effort from the realization of the conference to the completion of the book is highly valued. Ms. Sook Cheng Lim at World Scientific made a long trip from Singapore to Pasadena and the quality of the printed volume reflects her diligence and dedication to this project. Last but not least, I wish to thank Dr. K. K. Phua for his special care and enthusiasm for a prompt publication.

Ahmed H. Zewail
Pasadena, California
December 15, 2007

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The Preoccupations of Twenty-First-Century Biology

*David Baltimore**

The life sciences are in transition, with the beginning of the 21st century symbolically marking the initiation of this redefinition. It is a creative, exciting repositioning because it derives from a changed perspective on what is possible. The appearance of the human genome sequence at the turn of the millennium ushered in the post-genomic era, an era of new possibilities, new challenges and new paradigms.

Until the millennium, biologists struggled to produce data. Each of us had his or her particular perspective and each toiled to flesh it out by laboriously finding relevant proteins and genes, learning how they worked and trying to link them to the larger events of biological systems. Now we are reveling in a data and materials glut. All the genes and thus all the proteins are catalogued. Gene expression profiles in particular cell types are available online and comparisons of normal and diseased tissues are appearing daily. The sequenced genome in bite size pieces is readily accessed and an increasing fraction of the genome has been knocked out in available mice. And oh, the wonderful new machines we have to work with!

Consequently, life science research has become both easier and more difficult. Easier because we no longer have to clone a gene to get access to it; we can buy it from a supplier. If one wants to know something about a

*Division of Biology, California Institute of Technology, 1200 E. California Blvd., MC 147-75, Pasadena, CA 91125, USA, e-mail: baltimo@caltech.edu.

property of a protein, such as its structure, or one of a related protein, one can download it and study its molecular details. But the field is egalitarian: a scientist in India or California or London has the same access to data and materials. This makes originality harder to come by. Previously, one might have had a niche in biology to oneself, maintaining the hegemony by running ahead of the pack with a big laboratory of hard workers. But today such a strategy does not work because the access to tools and materials allows one to leap frog into the fray. Thus, there is a higher premium on innovative thinking, on the creative asking of questions.

The edge today goes to people who see a new avenue of application of the ubiquitous tool bag. One way to find open avenues is to focus on recently discovered molecules whose functions are up for grabs. The post-docs in my laboratory have increasingly been seeing such an avenue in microRNAs. Only found a few years ago and still in the process of being catalogued, the biological roles of these extraordinarily powerful little strands of RNA have yet to be extensively investigated, providing the post-docs with an open field. Actually, the field is only open temporarily; it is rapidly becoming crowded as more and more investigators ask how their pet area of study might be impacted by these previously unrecognized cellular components.

To study even a single microRNA, one uses all the tools of the moment: the array technologies, the data libraries, the sequence compendia. Mountains of data can be the investigator's friend when he or she approaches them from a well-defined point of view. But the data have stories to tell of their own. These stories are not about single entities but about relationships, correlations, interactions, control mechanisms, all of which can be teased from the data themselves. In the best of circumstances, the analysis leads to a hypothesis that can be tested by other data: data that may have to be collected. In this way, the data glut gets mined for significance and pathways of knowledge are unveiled. But more importantly, the new knowledge may suggest new perturbations that can be introduced into biological systems and then interrogated for their consequences. This back and forth between data, hypotheses, experimental design, collection of new data and then

verification or falsification of hypotheses is a rich and satisfying process, making contemporary biological science particularly powerful.

But this data mining and hypothesis construction is only a part of the 21st century scene. There is structural and mechanistic biochemistry, which benefits so greatly from the increased computing power we all enjoy. Protein folding, that tantalizing mystery at the core of biology, is also yielding to the power of digitalization and computation. Structural biology promises to finally deliver in this century its long-awaited fruits of precise knowledge of the molecular events that underlie the remarkable ability of linear combinatorial association of 20 amino acids to make the universe of nanomachines that carry out the processes of the biological world. And new and very expensive tools will give us insight into the aggregates of proteins that carry out the events of biology: the proteins of the synapse that send signals among neurons, the proteins of transcriptional control that give individuality to cells, the proteins of motors that distribute the contents of cells, the proteins of membranes that allow a cell to sense its environment.

It is a truism that the events of biology play out in time and time is the dimension hardest to resolve at the molecular and cellular level. It has felt like Heisenberg's uncertainty principle was at work: the better the spatial resolution, the less we know about the dynamics. In this arena, we are seeing the beginnings of a true revolution, with tools emerging that can give us spatial resolution measured even in nanmometers and still allow us to watch molecular events unfold. We can hope that the detailed events of biological catalysis will grace our textbooks in coming editions. Other techniques allow even single protein molecules or aggregates to be followed as they perambulate inside cells.

We must acknowledge that it is not only proteins that make up cellular life: lipids and carbohydrates — remembered from our textbooks but put aside by most researchers because they did not fit into the central dogma (even as modified by reverse transcription) — have stories to tell of specificity, control and interactions and these are just beginning to emerge.

They will surprise, with that surprise coming to us soon because they are drawing 21st-century attention.

What moves through the world is not proteins or carbohydrates or lipids: it is an organism, an integration of the molecules of life. In this early decade of the 21st century, there are stirrings of interest in integrative biology, usually under the rubric of systems biology and for metabolic questions, this, too, will yield over time to our computers, models and data collection. It will leave us with the toughest problem of biology facing us as the last frontier: the nervous system. Here we face integration on its largest scale, but an integration that can produce the ultimate emergent property: consciousness.

While cellular life outside of the nervous system will be unraveled and known in exquisite detail in the next few decades, the understanding of brains seems likely to tantalize biologists well into the century before a deep understanding emerges. But emerge it will and there will one day be sense to the obvious but presently incomprehensible statement that consciousness is the product of cellular behaviors.

And what will that leave us? What will be our concerns as the century ripens into older age? There will be concerns about synthesis. Just like chemists, we will prove that we understand biology by synthesizing it. And we will not just make over what evolution has produced through its slow, cumbersome four billion-year-long channeled meanderings, we will do it better. We will make things that evolution never got to. The power of gene manipulation will be not only to correct defects but also to create new capabilities. We will not be constrained by the usual constituents of cells. Carbon nanotubes and other nanostructures will interface with proteins providing new levels of control and readout. Medicine will not just be about curing but about replacing and ultimately enhancing.

As we come to the latter decades of our new century, our world will be very different. We will have harnessed the sun's energy directly, replacing the burning of stored chemical energy and watching a hot earth begin to cool back down: letting the universe slowly scour us of our poisonous gases.

Biology will have played a role there, somehow being the model for the capture and storage of the sun's energy. That will have been the conquest of the century. We will live long lives, battling disease to a standstill when it appears. We will lead enhanced lives, tightly linked to computers that will extend our range of activities and do much of the drudgery we now take on ourselves. We will still be humans of the same sort, fighting among ourselves but hopefully not with the guns that will have become so smart that no one can hope to outsmart them.

And we will continue to see vistas. Science will not come to an end because synthetic science knows no limits. There is always another structure to be made, another linkage to be created, another capability to add to our armamentarium. We will still be humans, still restless, still excited by the unrealized opportunity and committed to newness.

At least, that is the world I would hope to leave for those who come after me.

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The World as Physics, Mathematics and Nothing Else

*Alexander Varshavsky**

"There are two, and only two,
kinds of statements: trivial or incorrect."
(Lev Landau)

The title of this chapter would not surprise readers who have encountered articles by Max Tegmark about the foundations of physics and its relation to mathematics.[1,2] Practicing scientists — most of them anyway — know that science is one. The names of different sciences, be it "chemistry," "astronomy," "immunology," "linguistics," "psychology" and so forth, are shorthands that indicate the location of a specific domain of science *vis á vis* its other domains. In part because of the multiplicity of these names, the unitary nature and hierarchic structure of science tend to be less obvious to the nonscientists. This chapter covers, briefly, a lot of territory, and introduces a simple re-definition of terms that may improve the comprehension of scientific discourses by folks outside of science. It also pauses at philosophy and its discontents, and proposes a solution.

We begin with chemistry. In the 19th century, it was a common assumption that physics and chemistry were different in a profound way. This view was shown to be untenable when quantum mechanics accounted,

*Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA; Telephone: 626-395-3785; Fax: 626-440-9821; e-mail: avarsh@caltech.edu.

in the 1930s and later, for the properties of covalent and noncovalent interactions, and for the physical basis of the periodic table. At first, only the molecule of hydrogen (H_2) could be "explained," quantitatively, through quantum mechanics. Later, both conceptual and computational advances in a branch of physics called quantum chemistry extended this understanding to complex atoms and chemical compounds, including organic ones. In the course of these studies, there was never a reason to doubt that the receding possibility of doing exact calculations for larger molecules was caused solely by the (expected) increase in computational complexity, i.e., by nothing more mysterious than that. In other words, a statement that chemistry has been "reduced" to physics is correct in the well-defined sense of interactions that involve sufficiently small numbers of atoms, and is most likely to be also correct "in principle," for arbitrarily large molecules, their large ensembles, and their reactions.

This reductionism "in principle," as distinguished from "practice," underlies physics proper as well. The "*arrows of explanation*"[3] are far from random: they point, consistently, from complex systems, e.g., those encountered in solid-state physics, to systems with fewer and smaller components. These "arrows" eventually converge to the level of quantum mechanics of elementary particles and simple nuclei composed from them. An increase in complexity of a physical system often brings forth strikingly specific phenomena that are characteristic of larger systems and are not observed with their (sufficiently small) subsets. For instance, three molecules of hydrogen do not comprise a setting for which the concepts of temperature and pressure can be tangibly useful. But the Avogadro number or so of the same molecules more than suffices for the properties of temperature and pressure to become relevant. Examples of this kind are a legion.

High-level phenomena in the physics of many particles and the regularities — "laws" — that describe such systems are referred to as "emergent" qualities. "*More Is Different*" was the title of a 1972 paper by P. W. Anderson,[4] and more is different indeed, qualitatively so. Lev Landau was one of the first to suggest, in the 1950s (cited in Ref. 5), a

general idea that at least some emergent properties of a physical system are "generic" and characteristic of distinct stable states of the matter in the sense of being quasi-independent of the underlying "microscopic" laws. The resulting hierarchies of methods and approaches are tailored to a set of emergent properties of a system, and in most cases can afford to ignore the microscopic laws altogether. The underlying "fundamental" physics is still there, but often not in a way that has explanatory power or is helpful otherwise.

The hierarchic, "embedded" relationship between physics "proper" and chemistry can be extended, through the same logic, to biology, including molecular biology (a mix of biochemistry, biophysics, genetics and cell biology), neuroscience, and other "hard" or not so hard branches of biology, for example, psychology, sociology and linguistics. All three of the latter disciplines can be argued to be complex subsets of neuroscience, as they deal with behavioral, cognitive and societal aspects of humans, the only large-brained animals that possess recursive symbolic language and thereby are capable of communications and behaviors whose range and complexities vastly exceed those of other animals. A definitive proof that plants, animals, bacteria and other living organisms are nothing else but chemical (physico-chemical) machines of vast but finite complexities is not available in a formal sense. Until about the 1960s, a view (or rather a hope) was intermittently expressed that the understanding of biological systems may require fundamentally new physical "laws."[6] There is no current support for such views.

What I am about to suggest may seem radical only if it is (incorrectly) perceived as an assertion of a "truth." Instead, I propose, below, a set of *definitions* that have (as they must) the property of self-consistency but aspire to nothing greater than that. Specifically: let us call "physics" what is traditionally denoted as "science." "Science," in this context, would be simply a synonym of "physics." This definition addresses, in one step, the often encountered confusion, largely among nonscientists, about the multiplicity of "sciences," as if they are fundamentally separate entities. They

are not, and the above definition takes care of that. Physics "proper," then, would be defined as the one that deals with systems that are distinct from living organisms and encompass domains (high-energy physics, astronomy/cosmology, solid-state physics, applied physics, etc.) that are conventionally denoted as "physics." A particular subset of physics, called "chemistry," is defined as physical systems that involve large sets of atoms significantly above 0°K but at sufficiently low energies (temperatures) and pressures to enable their (reversible) interactions and formation of metastable atomic aggregates called molecules. That's chemistry. Biology, then — at least the biology known to us — is a particular subset of chemistry (and hence of physics) that involves temperatures between ~0°C and ~200°C, plenty of H_2O, carbon/nitrogen/phosphorus-based compounds, and complex (but finitely complex) entities, called cells, that have the properties of *replicators*, i.e., the ability to assimilate parts of media in which a replicator grows and to make (occasionally imperfect) copies of itself. The imperfection of copying results in *mutations* and leads to a quasi-random variation in the rate of propagation of replicator's progeny, thereby making possible the evolution, through "natural" selection (i.e., competition), among nonidentical replicators.

The extant cell-based replicators, while fundamentally similar in that they employ lipid membranes and DNA/RNA/protein-based machines, are also diverse in several respects. Numerically, most cells on Earth are prokaryotes. These cells lack a membrane-enclosed intracellular nucleus and do not (strictly) depend on multicellularity and division of labor among closely related cells. A subset of replicators called eukaryotes evolved to contain the nucleus, multiple chromosomes, and a range of other complex intracellular structures. Some eukaryotes became multicellular, with division of labor amongst the organism's cells and with intricate mechanisms that both repair the instruction tape (DNA) and recombine it with other (usually similar) DNA in germ-line cells specifically reserved for that purpose. This *genetic* recombination, largely through sexual reproduction and *meiosis*, is likely to be a form of repair/rejuvenation, i.e., the prevention of aging of a lineage of organisms related through descent. DNA recombination also

contributes to the ongoing competition with other organisms, including parasites. Prokaryotes are proficient too, in their own ways, in repairing their components, particularly DNA, and in carrying out DNA recombination, a process that also contributes to DNA repair.

While the evolution that led to this state of affairs was, to a large extent, the evolution through natural selection, a quasi-neutral mutational drift, referred to as *neutral* evolution, is also capable of producing increases in organismal complexity, and may have underlain, in part, the emergence of large multicellular organisms.[7–9] All of this is chemistry of course (i.e., a subset of physics), in the realm of complex assemblies of large and small molecules that are partitioned within enclosures made of lipid membranes, and function as replicators. As one might expect, these assemblies are fragile: even in the absence of overt insults, and especially upon them, cells employ roughly a third to a half of their entire machinery and "fuel" (ATP and related compounds) to keep undoing various errors and to repair, nonstop, their critical subsystems, with an emphasis on DNA. The resulting emergency-ward pandemonium (exacerbated by randomness of molecular collisions that underlie these circuits) includes the incessant repair of the repair devices themselves. All of these processes involve, among other things, a highly active, regulated turnover (destruction followed by resynthesis) of specific intracellular proteins.[10] Despite these remarkable efforts, most cells eventually die, through predation, aging (deterioration), or programmed cell death (apoptosis).

One lineage of mammals has yielded, over the last ~ 10^6 years, the only species that was capable of sophisticated (as distinguished from rudimentary) imitation, a skill that may have underlain the development, over the course of human evolution, of a syntax-enabled, vocabulary-rich language. This unique capability, in conjunction with other features of (evolved) human mind, led to the primacy of humans among mammals and other large animals. It also made possible the technological civilization, and underlies its continuing development. The emergence of language, the much later invention of writing, and the still later inventions of printing (15th century)

and electronic communications (20th century) have unleashed the *second species* of replicator, referred to as *memes*, by analogy with *genes*.[11,12] Memes are stories, songs, habits, religious beliefs, specific skills, and ways of doing things that can spread from person to person through imitation, either directly or through devices such as books, TV or the Internet. Memes, like DNA, can be thought of as information. DNA propagates from cell to cell, whereas memes, in a qualitatively distinct way (hence the *second* replicator) propagate from one mind to another meme-capable mind. DNA evolves without "intentional regard" for a vehicle (cell) that contains it. Similarly, memes, in their journeys from mind to mind, are free of "intentional concerns" about the minds that receive a meme and either spread it further, or hold it, or misremember ("mutate") it, or forget ("delete") it. The memetic evolution (a part of it is referred to as the evolution of culture) is much faster than a DNA-based one. Memes are thus a separate, profoundly distinct replicator. They did not exist before the emergence of human language, and now influence ("drive") the evolution of humans and their societies concurrently with DNA-based evolution, in complex and insufficiently understood ways. Potential ramifications of memes are fascinating (e.g., Ref. 12), but there is no credible reason to suspect that in discussing memes we might have left chemistry/physics behind. We did not. What we left behind are *complexity* levels of a discourse that underlies "conventional" chemistry, entering the field of a particular "high-level" chemistry, with its own "high-level" tools (they are still so-so, those tools).

Is medicine physics? Of course it is, as medicine is a branch of applied biology, and biology, as we already discussed, is chemistry/physics. The coming revolution in medicine will involve not only qualitatively better ways to do surgery, but also pharmacological (drug-based) therapies that will take into account, at last, the massive interconnectedness and redundancy of molecular circuits in living cells. Most of conventional single-compound (and even multi-compound) drugs of today are incapable of such finesse. Therefore even otherwise useful drugs exhibit undesirable side effects. Yet another problem is our continuing helplessness in containing

(let alone curing) major human cancers once they spread beyond a surgeon's knife. The problem is exacerbated by genomic instability of many, possibly most, cancers. This property increases the heterogeneity of malignant cells in the course of tumor progression or anticancer treatment and is one reason for the failure of most drug-based cancer therapies.[13,14] A few relatively rare cancers can often be cured through chemotherapy but require cytotoxic treatments of a kind that cause severe side effects and are themselves carcinogenic.[15,16] Recent advances, including the use of antiangiogenic compounds and inhibitors of specific kinases, hold the promise of rational, curative therapies.[17,18] Nevertheless, major human cancers are still incurable once they have metastasized.

I recently proposed an approach to cancer therapy that involves homozygous deletions (HDs). They are present in many, possibly most cancers, and differ from any other attribute of a cancer cell by the fact that an HD *cannot revert*.[19] Thus, a treatment that homes exclusively on cells that *lack* specific DNA sequences that are present in normal cells may be not only curative but substantially free of side effects as well. The difficulty here is that a homozygous deletion is an "absence," and therefore it cannot be a conventional molecular target. Nevertheless, an HD-specific anticancer regimen, termed *deletion-specific targeting* (DST), is feasible (on paper so far), at the price of relatively complex, "large" molecule-based designs.[19] If the complexity of DST-type strategies is unavoidable (that remains to be determined), approaches of this kind might be a harbinger of therapies to come. The virtues of simplicity notwithstanding, a complex problem, such as an assured cure of cancer, or a selective elimination of damaged (e.g., aged) mitochondria in cells of a patient, or other such feats may require commensurately sophisticated solutions. Can small compounds, with their inherently low informational content, ever enable a definitive cure of cancer that is also free of collateral damage? The notion that underlies (and motivated) the DST strategy[19] is that a curative, side effects-free treatment may require polymer-scale, multitarget, Boolean-type circuits, i.e., that simpler (smaller) drugs ultimately will not do, particularly in

regard to side effects. The task at hand is to address the validity of this assumption.

"*I heard a great deal, many lies and falsehoods, but the longer I lived the more I understood that there were really no lies. Whatever doesn't really happen is dreamed at night. It happens to one if it doesn't happen to another, tomorrow if not today, or a century hence if not next year. What difference can it make? Often I heard tales of which I said, 'Now this is a thing that cannot happen.' But before the year had elapsed I heard that it actually had come to pass somewhere.*" (From *Gimpel the Fool*, by Isaac Bashevis Singer.)

Did Singer write about Gimpel the Fool, or did he write about the origin of life? Our survey (a couple of paragraphs above) of biological evolution was about its stages *after* the emergence of membrane-enclosed replicators (cells) on a young Earth. This, largely Darwinian, evolution has been understood at least in outline. By contrast, the preceding stage, called prebiotic evolution, is a mystery: its potential routes appear to be insufficiently robust. We do not know the nature of the chemical paths that led to the first "really living" cells. We also do not know whether those cells were based on RNA or other macromolecules. One difficulty of working on this fiendishly intractable problem is that all traces of the first cells are, most likely, irretrievably lost. That means that even a construction of a "live" cell in a lab may not tell us exactly how life arose on Earth in the first place. Nevertheless, several heroic groups, notably Jack Szostak and colleagues, are at work to "*synthesize chemical systems capable of Darwinian evolution, based on encapsulation of self-replicating nucleic acids in self-replicating membrane vesicles.*"[20] The behavior of membrane vesicles that they discovered as a part of this effort is unexpectedly rich, and suggests potentially relevant prebiotic routes to the first cells.[20,21] Over the years, the proposed life-origin scenarios ranged from a steady accumulation of complexity[22] to a colossally improbable fluctuation that may happen, e.g., once in a lifetime of our (Hubble-volume) universe and gives rise, from scratch or nearly so, to a replication-competent autotrophic

cell ("autotrophic" denotes a cell capable of making its own components from simple compounds). The above fluctuation would be kind of inevitable if the universe (or, using the modern language, a multiverse; see below) contains an infinite number of stars and planets (e.g., Ref. 23). I return to the subject of multiverses near the end.

If the world is physics, what about philosophy? Richard Feynman's disdain for the subject would be obvious to anyone who read his Lectures on Physics: "*These philosophers are always with us, struggling in the periphery to try to tell us something, but they never really understand the subtleties and depths of the problem.*"[24] The beginnings of philosophy, in ancient Greece and even earlier, were synonymous with the emergence of science. Later, much later, specific subsets of science that had defined themselves through experiments and mathematics were escaping, in droves and for good, from philosophy's vagueness and play with words that often covered up the absence of content. A succession of parades, throughout the 20th century, by obvious charlatans like the Marxist philosophers in the former Soviet Union, the "intellectuals" like Jacques Derrida in France, or philosophers like him in other places, did nothing to increase respect for the subject amongst scientists who bothered to take a look at the writings involved. Some philosophers knew that their highfalutin word plays were "not even wrong." Their other brethren took their own gobbledygook quite seriously, and were, therefore, not charlatans through intent. But the net result, *vis à vis* the discerning world outside, was the same for both groups. There are (relatively rare) folks who call themselves philosophers (a good example is Daniel Dennett) but actually do what I would call science, neuroscience in the case of Dennett. I mention this to make sure I do not offend people who are not "philosophers," in my book.

The escapes from philosophy continue. One of the latest is the subject of consciousness. As recently as 50 years ago, this problem was a preserve of certifiable cranks, theologians and philosophers, who proclaimed (and the claim is extant as I write) that consciousness, in addition to being a difficult problem (very true), is unlikely to be solvable in the context of "reductionist"

science (how do they know?). Recent scientific work in this arena was led by Christof Koch, the late Francis Crick and other neuroscientists. The problem of consciousness was subdivided into subproblems. Some of them, including "*neural correlates of consciousness*," have become, by now, a legitimate part of neuroscience. In less than 3,000 years (if you think it's a long time, ask a geologist), the subject of consciousness went from a poorly articulated notion at a campfire in Mesopotamia, through a messy arena that sustained, fruitlessly, generations of theologians and philosophers, to the modern, partially successful attempts to define the issues sharply and rigorously enough, and thereby to enable experimentally testable (as distinguished from unfalsifiable) predictions. This kind of evolution is in store, I think, for just about every problem that is claimed to be a philosophical one.

I conclude with a conjecture about the nature of philosophy or, more accurately, with a reformulation of its definition *vis à vis* science. I find it odd that the "deepest" questions, for example the nature of causality, the nature of time, the nature of "reality" *vis à vis* "observers," the meaning (if any) of life, are kind of partitioned, in an ill-defined way, between physics and philosophy. The former is one of humanity's greatest accomplishments: it led the way, hand in hand with mathematics, in our learning of how to think more accurately and critically, so that we do not end up fooling ourselves the moment a question is even halfway subtle. In contrast, the philosophy, once "exact" sciences separated from it, has become a depressing sight. "*The history of philosophy is, by and large, the history of failed models of a brain*."[25] This assessment, harsh as it is, leaves out some of philosophy's other shortcomings, including its reliance on inherently ambiguous human languages.

"*If your horse dies, we suggest you dismount*."[26] This, in a nutshell, is what I propose to do. It is time to see that "philosophy" was a transient, scaffold-like enterprise, with a beginning and the end. The latter could already be glimpsed in the 19th century. The proposed reformulation is "constructive," in that it relegates all subjects of philosophy to specific branches of science. These branches, as we already saw, can be defined

(not "proven" to be, but *defined*) as subsets of physics. The reformulation can be stated in "operational" terms: suppose that one is presented with a "philosophical" statement that claims to have a specific, verifiable-in-principle content. Suppose, furthermore, that a close examination of that statement confirms that it is, indeed, likely to have "content". If so, my conjecture (and the resulting reformulation) is that the above statement can *always* be classed as belonging to science, not "philosophy." For example, a "content-positive" philosophical statement about the nature of mathematical proofs, or about human motivation, or about space-time can be viewed, without a stretch, as a statement in mathematics, in neurosciences, and in fundamental physics, respectively. Whether such "assignments" are always possible, and whether, as a result, the philosophy departments at universities are based on a long-term, unfortunate misunderstanding remains to be seen.

"*There are two, and only two, kinds of statements: trivial or incorrect.*" These words, by Lev Landau, are said to have been uttered by him fairly often, and not entirely in jest. He meant, for example, that mathematical proofs can be viewed as being, in a sense, tautologies,[27] irrespective of the subtlety or length of a proof. (The notion of tautology in mathematics transcends the more narrow meaning of "tautology" in everyday discourse.[27]) Landau also meant, more informally, that our statements about the world, if they are accurate enough to reflect the world's design — from planets and stars to humans — may be akin to proofs in mathematics, i.e., that they are "trivial" in a narrow, *nonderogatory* sense, and that all other, less accurate statements are simply irrelevant ("incorrect"). There is a connection, here, to a view of the world discussed, over the last decade, by several authors (Refs. 1 and 2, and references therein). It involves the assumption that mathematical objects are "discovered," not "invented." An antecedent of this view, referred to as Platonic, was known to the Greeks, and is a (mostly unstated) premise of many mathematicians. A vastly general perspective, referred to as Multiverse-IV,[1,2] is that the currently known physics (which can be formulated as a set of mathematical propositions) is an infinitesimally

small subset of all possible "physics," whose range encompasses, quite literally, all possible mathematics. (Multiverses I to III are models that do not go as far as Multiverse-IV.[28,29]) Viewed this way, the seemingly extreme classification that I began with, i.e., that everything is physics, was quite timid. The proposed unification of physics and mathematics[1,2] would account for the old puzzle of the "*unreasonable effectiveness of mathematics in physics*,"[30] as mathematics and physics would be, in that view, the same thing. Hence the title of the present chapter.

These and related concepts, widely discussed by cosmologists, include the possibility that *nothing* determines the choice of vacuum state for a Hubble-type universe. (See Refs. 1 and 31 for the definitions of a vacuum state and a Hubble volume.) In this view, the universe we inhabit is an infinitesimally small speck within a multiverse that contains every possible type of a vacuum state. If so, then, for example, the electron-to-proton mass ratio, a fundamental constant in the observable cosmos, would be just a (variable) parameter, akin to the distance between a star and its planet. Both numbers would be determined by stochastic events ("historical" accidents) that attended the formation of this or that big-bang universe.[1,31] The values of "fundamental" parameters would seem to be "restricted," then, solely by the fact that we, the "observers," must be present to measure the properties involved, i.e., that life, let alone "intelligent" life, requires "accommodations," e.g., the properties of a universe that are compatible with the existence of (relatively) stable atoms, stars and planets. Thus, the laws of physics (and consequently of everything else) that we register by studying physics in our part of the cosmos may be determined not necessarily by fundamental principles but instead by the contingency of having to have an "observer" in place to perceive those laws. This notion is referred to as the anthropic principle.[31–33] At first sight, these concepts, including Multiverse-IV,[1,2] are far-fetched enough to be untestable and therefore would forever belong to "metaphysics," a set of unfalsifiable speculations. Recall, however, that the distinction between physics and metaphysics is determined by whether a conjecture in question is testable at least in principle, and not by whether

that conjecture is bizarre or involves currently unobservable entities. As discussed by others (Refs. 1, 2, 31 and 34, and references therein), both the anthropic principle and specific models of multiverses have explanatory powers, and may become a part of mainstream physics. Ramifications of these remarkable concepts and conjectures are so much beyond my mettle that this would be a good place to stop.

Acknowledgments

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chapter 3

Physical Biology
4D Visualization of Complexity

*Ahmed H. Zewail**

The integration of physics and chemistry with biology — physical biology — offers new opportunities for the deciphering of its complexity. Because of the collective interactions of the many elements involved, 4D visualization of structural dynamics is essential to an understanding of the mechanism of the function. Here, we provide an overview of the principles of 4D visualization and highlight the potential of space–time imaging through some examples of applications, ranging from chemical reactions and phase transitions to molecular assemblies and biological cells. Some "big questions" are raised in the hope that the new tools and concepts will provide an understanding of what complexity, and emergence, actually mean.

1. Prologue

The aim of physical biology as a new discipline is, as the title of the book implies, the integration of physics and chemistry united to explore the complexity of biology. Understanding mechanistically how physical forces and interactions govern biological function, from the molecular to the cellular scale, uncovers the nature of microscopic processes such as protein folding/misfolding, self-assembly and order, and the unique function of life's matrix, the triatomic water in cells. On the other hand, the study of information flow and circuitry of the cell provides the possibility for painting maps of interactive elements which are important especially in

*Physical Biology Center, Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, CA 91125, USA, e-mail: zewail@caltech.edu.

the programmatic diagnoses of diseases in medicine. In physical biology, the focus on physical methods and concepts for elucidating the complexity of structures and dynamics involved is distinct from the aim of mapping the engineering of information flow; i.e., the networks or wirings in cells, systems biology.

Because the elements of biological machines are defined on the scale of macromolecules, one is concerned with how they interact, communicate, and define a nanometer-scale function. This machinery derives its power from the control it exerts, with atomic-scale precision, which is responsible for the so-called emergence. Emergence is a new addition to the lexicon of biology, and other fields, but its precise definition is still amorphous. Given that biological machines operate in the nonequilibrium state, irrespective of viewpoint, we have to understand how the pieces are made, how their

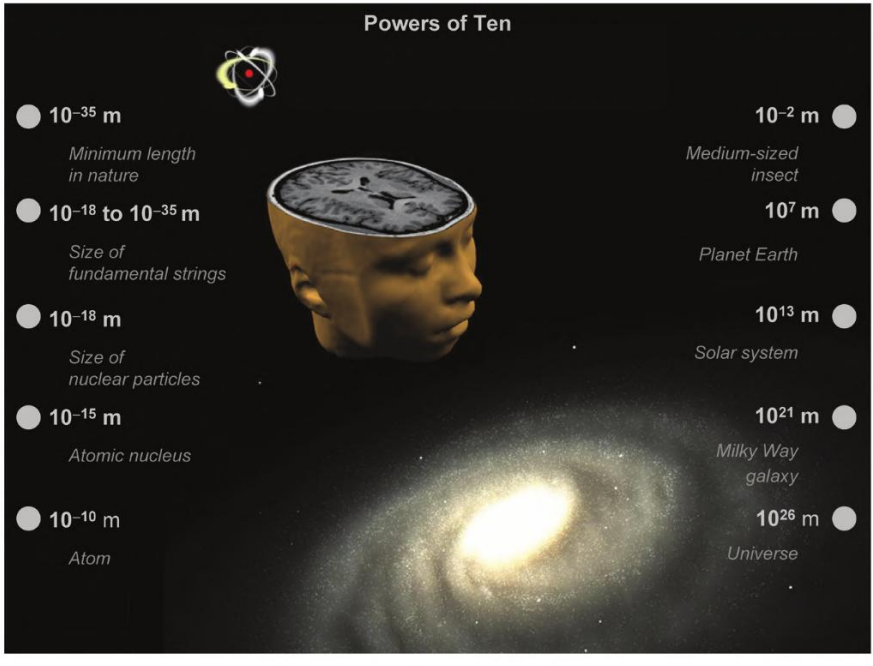

Fig. 1. Scales in powers of ten, from the atom to the universe.

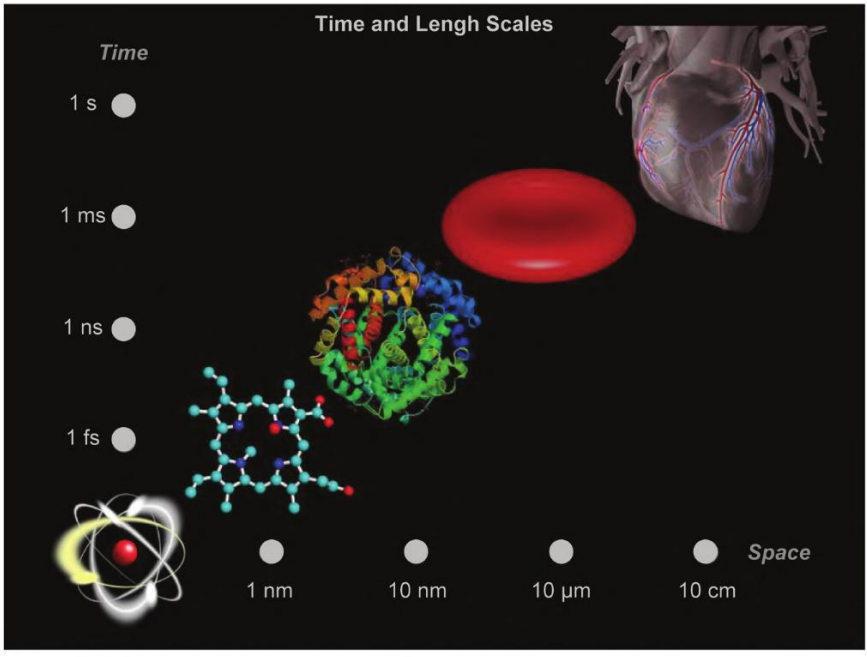

Fig. 2. Time and length scales, from atoms to organs. Shown are schematically represented atom, heme molecule, hemoglobin, red blood cell, and a heart.

physical forces exert control, and how feedback and feedforward of elements allow for function and robustness of information flow.

The emerging disciplines of physical, systems, and synthetic biology are clearly exciting frontiers in this century. For all, understanding mechanisms would remain dark and elusive, or at best speculative, unless the processes involved are directly visualized. The goal is to dissect complexity by following the behavior (function) in the four dimensions of space and time, of structure and dynamics. Such an understanding must account for the unique features of complexity — selectivity, diversity, directionality, and organization — and for the molecular forces controlling function. The mechanism may be "reduced" to elementary steps using the language of atoms (quantum mechanics), or we may need new concepts that tell us why

the "whole is greater than the sum of its parts" and why coherence and order are possible under the random thermal conditions of a physiological temperature.

2. Visualization: From the Very Small to the Very Big

Realization of the importance of visualization and observation is evident in the exploration of natural phenomena from the very small to the very big. A century ago, the atom appeared complex, a "raisin or plum pie of no structure," until it was visualized on the appropriate time and length scale. Similarly, with telescopic observations a central dogma of the cosmos was changed and complexity yielded to the simplicity of the heliocentric structure and motion in the entire solar system. From the atom to the universe, the length and time scales span extremes of powers of ten (Fig. 1). The electron in the *s*-orbital of hydrogen has a "period" of sub-femtosecond, and the size of atoms is on the nanometer scale or less. Our universe's lifetime is ~13 billion years and, considering the light year ($\sim 10^{16}$ m), its length scale is on the order of 10^{26} m. In between these scales lies the world of life processes with scales varying from nanometers to centimeters and from femtoseconds to seconds (Fig. 2).

For the atom, not only was the language (quantum mechanics) developed but also the behavior was controlled — it has essentially been tamed. Since the first conceptual idea that "*there are only atoms and the void*" postulated by Democritus more than two millennia ago, we now know the components of the atoms and how to detect them, count each one, and cool them to sub-kelvin or trap them with light. Of major impact in molecular sciences is the ability to observe atoms at rest at angstrom resolution and atoms in motion at femtosecond resolution. However, for macromolecules, complexity arises from the collective interactions of thousands of atoms to form structures as well as from their dynamics, which determine the functions and the rates of such functions.

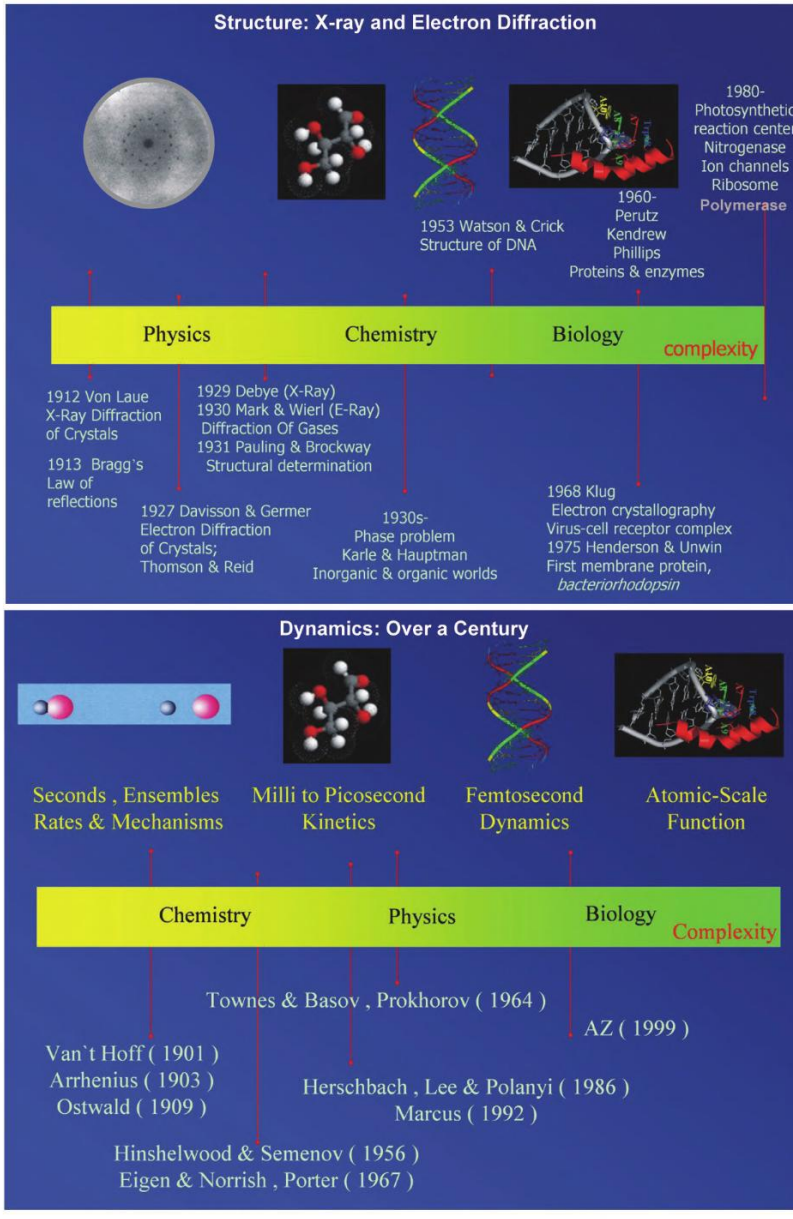

Fig. 3. (*Top*) Highlights of X-ray and electron diffraction determination of structures. (*Bottom*) Milestones, over a century of developments, in dynamics.

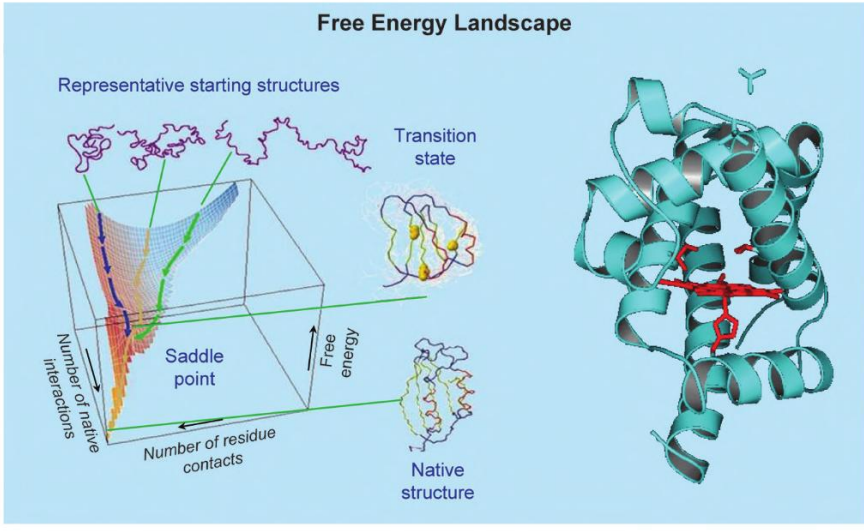

Fig. 4. (*Left*) Free energy landscape; see the chapter by Dobson. (*Right*) The structure of myoglobin.

Over a century of developments, X-ray and electron techniques have led to structural determination, beginning with the structures of two atoms (sodium chloride) and now culminating in the structural determination of more than 10^5 atoms in molecular mechanics. Similarly, for dynamics, the time scale at the beginning of the 20th century was practically in the seconds (hydrolysis of sugars); now, it has reached the atomic scale of femtosecond (Fig. 3). In the early days of DNA structural determination (1950s), it was believed, "*If you want to know the function, determine the structure*," a statement made by Francis Crick that dominated the thinking of the time. But as we learn more about complexity, it is clear that the so-called "structure-function" correlation is insufficient to establish the mechanisms in complex systems. An example that may illustrate this point comes from the function of proteins.

Both the structures of the hemoglobin and myoglobin have been determined, but we still do not understand how they fold, how they

selectively recognize oxygen, how oxygen is liberated from the cage into the medium, and how the matrix water assists folding, and the role it plays in directionality, selectivity, and recognition. Measuring the timescales involved through spectroscopic probes does not provide the structural transformations describing the complex energy landscape and involving many nuclear motions (Fig. 4). Visualization of the changing structures during the function is what is needed. The wavelength of the probing radiation or particle must be on the scale of interatomic separations (sub-nm), and for this reason, X-rays and electrons are ideal. However, if used as diffraction techniques in Fourier (reciprocal) space, then an inversion methodology is needed to deduce the structure in real space.

3. 4D Electron Microscopy

Our effort at Caltech in recent years has focused on the development of 4D visualization with ultrafast electron microscopy; development of electron diffraction has also been part of this effort. Electron microscopy provides real-space direct imaging with atomic-scale resolution. Here, I briefly highlight the concept and a few recent examples from physics, chemistry, and biology (the article by Sir John Thomas in this volume provides a lucid and comprehensive overview of the revolutionary advances in microscopy and imaging). The questions of interest, which are pertinent to physical biology, are numerous and include the following few: How do the elements of a polymer molecule of thousands of atoms, following the rules of genetic expression, interact to render a native structure capable of a specific function? Why, when they misbehave, do we contract diseases such as Alzheimer's? and Why do atomic-scale conformational changes of some macromolecular systems, as in prions, give rise to diseases?

The essential concept in 4D ultrafast electron microscopy (UEM) is based on the premise that trajectories of coherent and timed single-electron packets can provide the equivalent image obtained using *N*-electrons in conventional microscopes (Fig. 5). Recently, this goal of obtaining real

images and diffraction patterns using timed, single-electron packets was achieved. In the new design, a femtosecond optical system was integrated with a redesigned electron microscope operating at 120 keV or 200 keV. By directly illuminating the photocathode above its work function with extremely weak femtosecond pulses, both real-space images and diffraction patterns can be obtained. A high-frequency train of pulses separated by nanosecond or longer intervals allows for the recording of the micrographs in a second or so (see below). Single-electron imaging circumvents the repulsions between electrons (space-charge) and the concomitant decrease in the ability of the microscope optics to focus electrons, as well as to provide the necessary

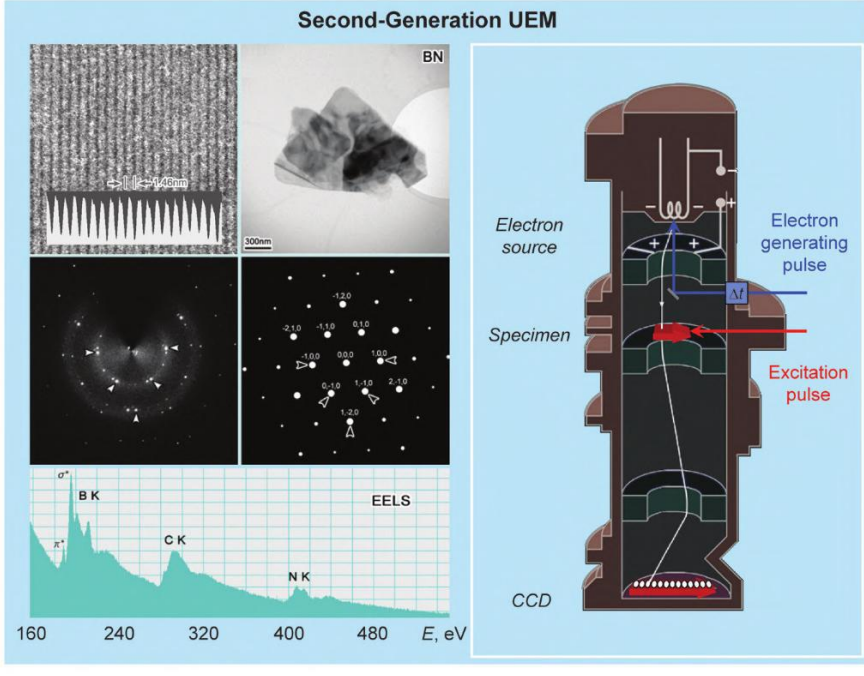

Fig. 5. (*Left*) Microscopy image (atomic scale), diffraction patterns (atomic resolution), boron image map, and electron energy loss spectrum, all taken with Caltech's 200 keV UEM. (*Right*) Single-electron trajectory schematized in UEM; see text and references.

stability of the electron flux during image processing. Depending on the required spatiotemporal resolutions, UEM can, in principle, operate in two modes: single-electron and single-pulse imaging, and the energy spread can be minimized by controlling the excess energy above the work function and/or invoking reverse-chirp methodology (see references). With frame-referencing at different times, the sensitivity of change is optimal for the isolation of transient structures.

At the atomic-scale resolution, we had to consider the length and time scales of electron imaging in UEM, namely, the energy-time and space-time relationships. Of particular importance are the fundamental limits of fermionic electrons in determining the coherence volume in imaging and diffraction. These issues have been discussed elsewhere, but suffice it to mention here that unlike bosonic photons which can occupy the same space over the duration of a coherent pulse, the Pauli exclusion principle restricts the volume in phase space for electrons. In general, the phase space can be thought of as divided into cells and electrons belonging to this volume element are indistinguishable; if more than one electron can occupy the volume, statistics is then used to deal with the problem. On the other hand, if the microscope operates in the "dilute regime," i.e., less than one electron per cell, then the "degeneracy parameter" is much less than one and the problem can be considered without yielding to statistics. The analogy with light (bosons) is useful, considering that the degeneracy factor is about 10^{-4} for a blackbody radiation (incandescent source at 3000 K and frequency of 5×10^{14} Hz), but about 10^{9} for a common helium-neon laser.

The coherence volume, with a quantum size proportional to $\hbar^3$, is related to the classical value which can be calculated from a knowledge of the speed (or wavelength λ) at which the accelerated electrons pass the specimen, the spread in the electrons' energies, and the ratio of λ/α, where α is the divergence angle of the source. Using typical values for a 120 kV acceleration (λ = 3.348 pm), the volume is obtained to be about 5×10^{5} nm^3. For our second microscope operating at 200 kV, it approaches 10^{7} nm^3 (10^{-14} cm^3) at λ of 2.507 pm. In the single-electron pulse mode, the number

of electrons per cubic centimeter is 10^{12}. Thus, the degeneracy factor is orders of magnitude less than one and each electron interferes with itself! In real space, and for a given contrast, the spatial resolution is only limited by the number of electrons.

4. Applications of 4D Electron Imaging

The space-time resolutions, and sensitivity, provide the impetus for investigating diverse dynamical phenomena of complex molecular, cellular, and material structures. Some examples from different fields illustrate the scope of applications.

4.1. Physics

Structures of nonequilibrium phases, which are formed by collective interactions, are elusive and less explored as they are inaccessible to conventional studies used for the equilibrium state. In order to understand the nature of these optically-dark phases, it is important to observe changes in structure at atomic-scale resolution. Such direct observations were recently made for the superconducting cuprates. The specific material studied is oxygen-doped LCO ($La_2CuO_{4+\delta}$); the undoped material is an antiferromagnetic Mott insulator, whereas doping confers superconductivity below 32 K and metallic properties at room temperature. Following near infrared pulse initiation, a structural phase transition was observed, defining a nonequilibrium state that is born in ~30 ps and lasts for ~300 ps with a major structural change along the *c*-axis of the material. Perhaps one of the most striking findings is the correlation found between photon and carrier doping (at the superconducting level); see references section. But a more pertinent example is that of metal-insulator transitions, which are common in the transformations of solid-solid materials and serve as an example of complex structural dynamics studied by both UEM and ultrafast electron crystollagraphy (UEC).

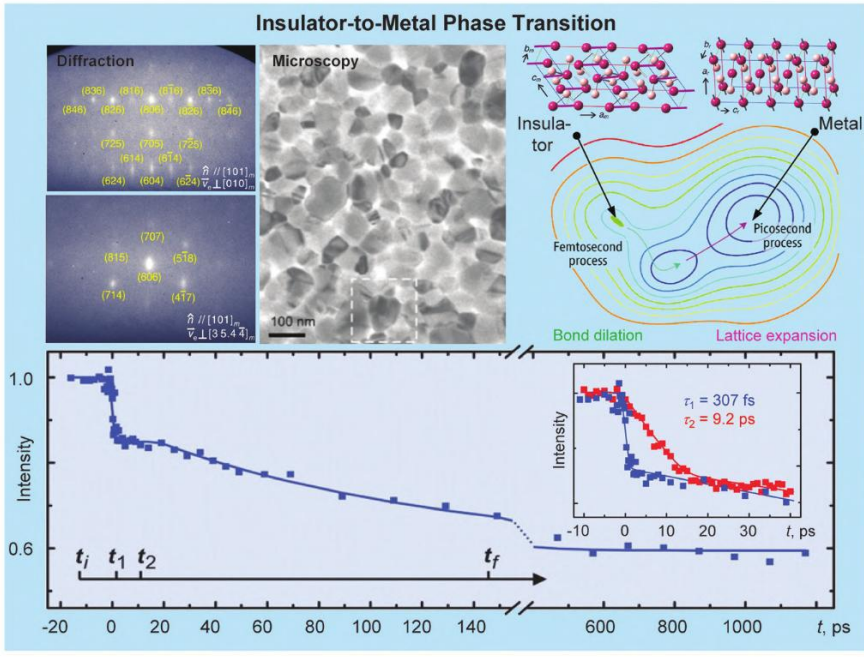

Fig. 6. Insulator-to-metal phase transition in vanadium dioxide. Equilibrium structures are shown, together with dynamics of atomic motions, as studied by UEM and UEC.

The physics of vanadium dioxide structure is a prototypical case. This room-temperature insulator has a monoclinic structure. Pairing and tilting of the vanadium pairs along one axis (Fig. 6) is at the heart of the insulating behavior. Part of the glue that keeps the paired atoms close to each other comes from the localization of two electrons on the vanadium sites, and from the gain in exchange energy when spins point in opposite directions. Excitation removes a fraction of these electrons and weakens or dilates the bonds, which is sufficient to unleash a collective relaxation of the structural distortion, a delocalization of the charge, and a loss of magnetic order.

Deciphering the complexity of motions became possible when mapping in time was made at once for different Bragg interferences (spots). The most important information comes from the observation that the fastest atomic

motions during the change occur along one particular axis of the crystal, and are related to pairs of vanadium atoms moving apart from one another (Fig. 6). Only at later times do the other crystallographic planes expand. The transformation pathway between stable monoclinic and tetragonal phases was shown to pass through an unstable, tetragonal unit cell, which is compressed along one of the axes, thus establishing a direct connection between the femtosecond dilation of the V–V bond and the equally fast changes in conductivity that can be measured using conventional techniques. For the same material, UEM images (Fig. 6) were obtained for nm-scale crystallites, showing the influence of connectivity and carriers density on the transformation.

4.2. Chemistry

Because of the strong interaction of electrons with matter, one of the advantages of using electron imaging is the ability to study chemical reactions under collisionless conditions, and at nm-scale molecular interfaces. A "textbook" case for the former is that of the non-concerted elimination reaction of haloethanes (ICF_2CF_2I). The methodology of using different timed pulses in ultrafast electron diffraction (UED) allows for the isolation of the structure(s) of the reactant, intermediates-in-transition, and the product. The specific reaction studied involves the elimination of two iodine atoms from the reactant (haloethane) to give the product (haloethylene). Because of its fleeting nature, the structure of the intermediate has not been determined previously, and the challenge was in determining the structural dynamics of the entire reaction. This was achieved by referencing the observed diffraction to well-isolated frames at different times. In this way, we determined the bond distances and angles (Fig. 7). Moreover, the molecular structure of the $CF_2CF_2I^{\cdot}$ intermediate was established from the frame referencing at a time after the femtosecond breakage of the first bond. For other systems, reactions in the ground and excited states were similarly studied under collisionless conditions, in the absence of solvent perturbation (see references).

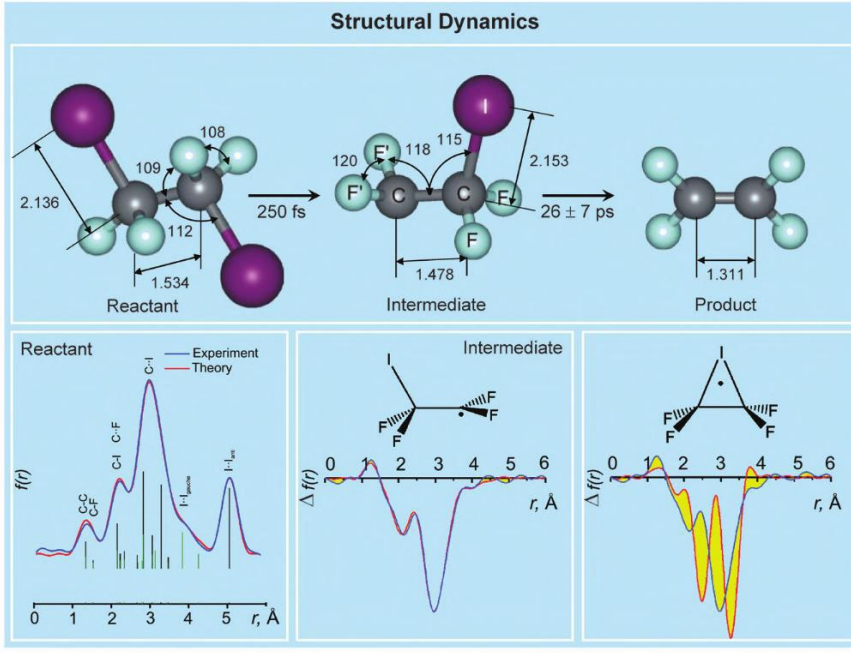

Fig. 7. Structural dynamics of the indicated chemical reaction, with the reactant, intermediate, and product structures determined under collisionless condition.

At interfaces, molecular assemblies, such as water networks, are unique in their behavior. Surface water of cells has unique properties that are distinct from those of liquid water, while amorphous ice, which constitutes the bulk of matter in comets, has very different properties. The directional molecular features of hydrogen bonding and the different structures possible, from amorphous to crystalline, make the interfacial collective assembly of water on the mesoscopic scale much less understood. Structurally, the nature of water on a substrate is determined by forces of orientation at the interface and by the net charge density, which establishes the hydrophilic or hydrophobic character of the substrate. However, the transformation from ordered to disordered structures and their coexistence must depend on the time scales for the movements of atoms locally and at long ranges.

Therefore, it is important to elucidate the nature of these structures and the time scales for their equilibration.

This problem of interfacial water was addressed by determining both the structure and dynamics using hydrophobic or hydrophilic surface substrates. The interfacial and ordered (crystalline) structure was evident from the Bragg (spots) diffraction and the layered and disordered (polycrystalline) structure was identified from the Debye-Scherrer rings (Fig. 8). The temporal evolution of both phases, after the temperature jump, was studied with monolayer sensitivity. On the hydrophilic surface substrate, the structure is found to be cubic (I_c), not hexagonal (I_h), and on the hydrophobic surface, the structure remains cubic, but very different in the degree of order.

Structural dynamics is different for the two phases. Of special interest is the influence of interface on the coexistence of these structures, and the time scales of energy transfer and disruption of the hydrogen bond network.

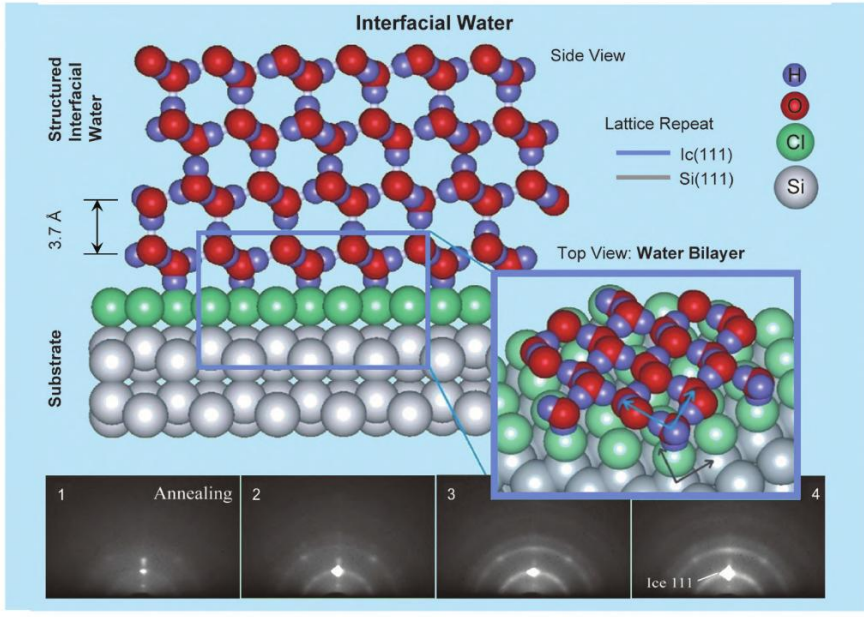

Fig. 8. Structural dynamics of interfacial water.

At the microscopic level, several conclusions were drawn. First, the reaction coordinate for breaking hydrogen bonds involves a significant contribution from the O···O distances, as evidenced in the depletion with time of the corresponding peak in the radial distribution function. Second, the time scale of energy dissipation in the layered structure must be faster than that of desorption, as no loss of water molecules was observed. Third, the time scale of the dynamics at the interface is similar to that of water at protein surfaces. Finally, the order of water molecules at the interface is of a high degree. Using molecular dynamics, in collaboration with Parrinello's group, the nature of forces (the atomic-scale description) and the degree of order were examined, elucidating features of crystallization, amorphization and the time scales involved. There are still questions pertinent to the topology of the energy landscape, spatial voids at the interface, and energy transfer from the substrate. Work is continuing on this problem which is relevant to other fields, including biology.

4.3. Biology

As mentioned earlier, biological complexity is a continuum of changing length and time scales. Among the phenomena of strongly correlated systems, self-assembly and self-organization are two that exemplify the controlled molecular and nonlinear interactions — physics and chemistry join in here! The simplest of assembled membrane-type structures is a bilayer of fatty acids. These long carbon chains self-assemble on surfaces (substrates) and can also be made as "2D crystals." To achieve crystallinity, the methodology of Langmuir–Blodgett films is invoked, providing control over pH, thickness, and pressure. In our studies, a temperature-jump of the substrate was introduced to heat up the adsorbed layers in direct contact with either a hydrophobic or a hydrophilic substrate. This initiating femtosecond infrared pulse has no resonance for absorption to the adsorbate layer(s). The studies made for monolayers, bilayers and multilayers of fatty acids and phospholipids provided an opportunity to determine the structural

dynamics at the interfaces of nanometer scale and to examine changes due to the transition from 2D to 3D dimensionality.

The transient anisotropic expansion of fatty acid and phospholipid layers is vastly different from that observed in the steady state at equilibrium. On the ultrashort time scale, the expansion is along the $-CH_2-CH_2-$ chain, but the amplitude of change far exceeds that predicted by (incoherent) thermal expansion. If heating is that of an equilibrated system, the change in the value of c_0, the unit cell length along the chains, with temperature, should be independent of the number of $-CH_2-CH_2-CH_2-$ sub-units in the chain, as thermal expansion determined by the anharmonicity simply gives $\Delta c_0/c_0 = \alpha$, where α is the thermal expansion coefficient, typically very small, $10^{-5}\,K^{-1}$. For a 10-degree rise, this expansion would be on the order of 10^{-4} Å, while the observed transient change is as large as 0.01 Å. The in-

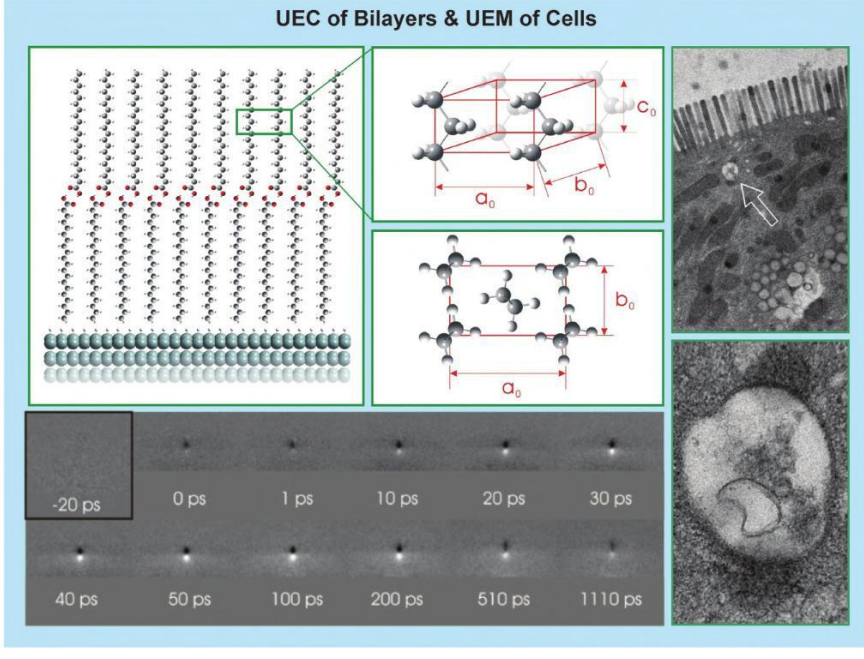

Fig. 9. Bilayers and cells studied by UEC and UEM.

chain large amplitude of expansion is understood even for harmonic chains, provided the system is in the nonequilibrium state, as detailed elsewhere (see references and also Fig. 9).

The impulsive force at short times transmits a large change in the value of c_0 as the disturbance (wave-type) accumulates to give the net effect that is dependent on the number of C atoms. In other words, as the disturbance passes through the different bonds, the diffraction amplitude builds up and exhibits a delay, ultimately giving rise to a large total amplitude for the change. This picture also explains the dependence of expansion on the total length of the chains, the increase in the initial maximum amplitude as the temperature of the substrate increases, and the effect of substrate strong (hydrophilic) vs. weak (hydrophobic) binding. The fact that the initial change in intensity (and elongation) occurs on the 10 ps time scale and that the distance traveled is ~20 Å (for a monolayer), the speed of propagation should be sub-kilometers per second, which is close to the propagation of sound waves. The speed could be of a higher value, reaching the actual speed of sound in the layers, a ballistic propagation in the chains. The model of coherent coupling among bonds in the underdamped regime of harmonic motions yields results vastly different from those of the diffusive behavior in the overdamped regime, but it has some common characteristics to the Fermi–Pasta–Ulam model of anharmonically-coupled chain dynamics. Preliminary MD results show the increase in $-CH_2-CH_2-CH_2-$ distance near the silicon surface by 0.08 Å in about 5 ps. With the same approach, studies of the self-assembly were made to elucidate the formation of interchain stacking with the void channels in between at zero pressure. Further research in this area will explore the role of hydration, and the extension to ion channels.

Biological imaging has the potential of producing structures for thousands of complexes and conformations important in cell biology. Cryoelectron microscopy (cryoEM) is an essential part of this endeavor. In the "single particle analysis" technique, purified proteins are spread into thin films across EM grids, plunged into liquid ethane to immobilize the proteins within vitreous ice, and imaged in an electron microscope. The

key advantages of cryoEM-based methods are several. First, because the proteins are imaged one-by-one, in isolation rather than in a crystal, no time-consuming searches for crystallization conditions are necessary; second, the proteins can be imaged in physiologically relevant buffers and concentrations rather than those special conditions that produce crystals; and third, a mere $\sim 10^8$ particles are estimated to be needed to determine the structure.

Despite steady progress, the highest resolution achieved by single particle cryoEM is 4–8 Å, but typical structures are still determined at a resolution of 1–2 nm. The limit on resolution is not fundamental as the current resolution is determined by radiation damage among some other factors, such as particle heterogeneity and image contrast analysis. High energy electrons break covalent bonds, deposit thermal energy, and occasionally even knock out atomic nuclei. Thus, radiation damage limits the useful dose to $\sim 20\ e^-/Å^2$, a flux issue. Because near-atomic resolution reconstructions require pixel sizes of approximately $1\ Å^2$, the quantum noise ("shot noise") present in the images makes it difficult to align the images precisely; misalignment directly degrades reconstruction resolution.

However, a major consequence of radiation damage is the movement of specimen during the exposure, which causes blurring of images. At about 80 K, proteins in vitreous ice are essentially motionless. Nevertheless, when hit by an electron beam, and covalent bonds are broken, electrons are liberated and heat is deposited in the macromolecule and surrounding ice. Pressure builds up within the ice as radiolytic fragments move at least a van der Waals radius apart. Residual positive charge accumulates as secondary electrons (electrons originally present in the sample) are emitted, causing internal repulsions. As a result, the deteriorating macromolecule might move and rotate appreciably. By reducing the exposure times by many orders of magnitude (from $\sim 10^0$ to somewhere between 10^{-6} and 10^{-12} seconds) beam-induced specimen movement becomes negligible on the time scale of the UEM experiment. This concept is currently under experimental scrutiny in a collaborative effort with my colleague Grant Jensen.

Three significant features of biological UEM are noteworthy. First, by dramatically reducing specimen movements, the resulting images should be much sharper. Sharper images will not only contribute more accurate information to 3D reconstructions, but will also allow for information to be merged correctly because the images are alignable with higher precision. This advance may make single particle analysis with near-atomic resolution a common methodology. Second, while this "freezing-in-time" concept is important to imaging of single (i.e., noncrystalline) macromolecules, UEM also opens the possibility of recording femtosecond (or longer) time scale dynamics. Thus, it is conceivable, for instance, to obtain time frames of single particles after an excitation pulse warms the sample, excites a conformational change, or releases a photocaged reactant. There are no fundamental physical reasons why multiple electron pulses could not be configured to simultaneously record whole tilt series, producing dynamically-resolved 3D tomographs of particles or cells. Finally, in biological UEM the regularity of pulsed dosing may result in the control of energy redistribution and heat dissipation and such controls are currently under experimental examination.

With UEM, images of cells derived from the small intestines of a four-day old rat were obtained. The specimen was prepared using standard thin-section methods. The cells were positively stained with uranyl acetate causing them to appear dark on a bright background. Figure 9 depicts the UEM images of the cells at two different magnifications. These images were obtained using the pulse trains of UEM with an exposure time of a few seconds; such exposure times compare well with standard EM imaging. In the figure, both the microvilli and the sub-cellular vesicles of the epithelial cells are visualized.

The images and diffraction patterns discussed above for materials and biological cells are "snapshots" or "frames" at a particular point in time. However, by delaying a second initiating optical pulse to arrive at the sample in the microscope with controlled time steps, we obtained a series of such snapshots with a well-defined frame time (movie). So far, such movies

have been made for materials exhibiting a first-order phase transition (see references) using the UEM1 apparatus. More recently, we have developed UEM2 with additional capabilities for resolving the electron kinetic energy and for scanning the electron probe. In this UEM2, atomic-scale spatial and energy resolutions were achieved (Fig. 5). The latest in these studies is the success in UEM recording of protein single crystal (catalase) images, making visible in real space the lattice separations of 6.85 and 8.75 nm. We have also achieved, in UEM-2, a new limit of resolution, being able to visualize the 3.4 Å separation of a graphitized specimen, without the field emission gun (FEG) required in conventional (atomic-scale) electron microscopy. Moreover, the number of electrons has now been increased to permit bright- and dark-field scanning UEM.

4.4. Nanoscale Mechanical and Melting Phenomena

Another dimension of UEM is that of the *in situ* study of mechanical, melting or crystallization phenomena on the nm scale. Recently, we reported the serendipitous discovery of a mechanical nanoscale molecular phenomenon, a switchable channel or gate (Fig. 10) observed in a material of crystalline quasi-one-dimensional (1D) semiconductor Cu-TCNQ (TCNQ = 7,7,8,8-teracyanoquinodimethane, $C_{12}H_4N_4$). Remarkably, the switching, after a shock, not only is reversible with the near infrared pulses being on or off, but also returns the material in space to the original structure. The functional behavior is robust in the relatively low-fluence regime. At significantly higher fluences, we observed, in the microscope, the internal dilation and the reduction of the copper ions to form islands of neutral copper metal structures.

The strong electron acceptor (π-acid) TCNQ undergoes a facile redox reaction at room temperature with metals such as silver and copper. In the single crystals of the resulting Cu-TCNQ charge-transfer complex, Cu^+ and $TCNQ^{-\cdot}$ form discrete columnar stacks in a face-to-face configuration with strong overlap in the π-system. Further, the copper atoms are bound in a

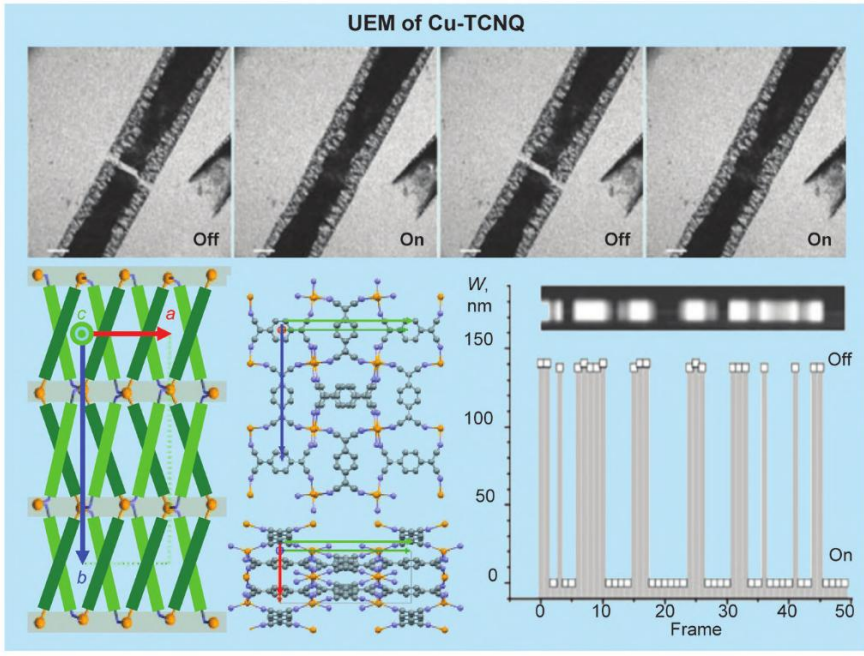

Fig. 10. Nanoscale mechanical phenomena observed by UEM in the quasi-1D Cu-TCNQ material. The nanogate width is denoted by *W*.

four-coordinate, highly distorted tetrahedral geometry to the nitrogen atoms on the cyano groups of the TCNQ molecules. The strong through-space interactions between the π-electrons and the resulting quasi-1D structure of the material in the solid state impart interesting structural and electronic properties in the field of the low-dimensional organic solids, and in the exploration of one-dimensional semiconducting nanostructures.

Not only is the controlling excitation the key to gating of the channel at lower fluences, but also it is sufficient at 1.6 eV to facilitate charge transfer and hence weakening of copper bonding and enhancement of its mobility at higher fluences. The surface energy of metal clusters composed of a few atoms is quite large, and there is a strong driving force for the smaller clusters to coalesce (Ostwald ripening). This behavior greatly

enhances the formation of larger copper clusters and further reduces the ordered structure of the Cu-TCNQ material until finally, at relatively high fluence, the crystal separates into its constituent atomic and molecular components. The results discussed herein for channel formation and reductive metal clustering open the door to numerous further studies, including those of nucleation, charge/energy transport, and ultrafast dynamics of dislocations and coherent nuclear motions. The findings may be of value in applications involving molecular nanoswitches and channels, as well as optical pulse memory.

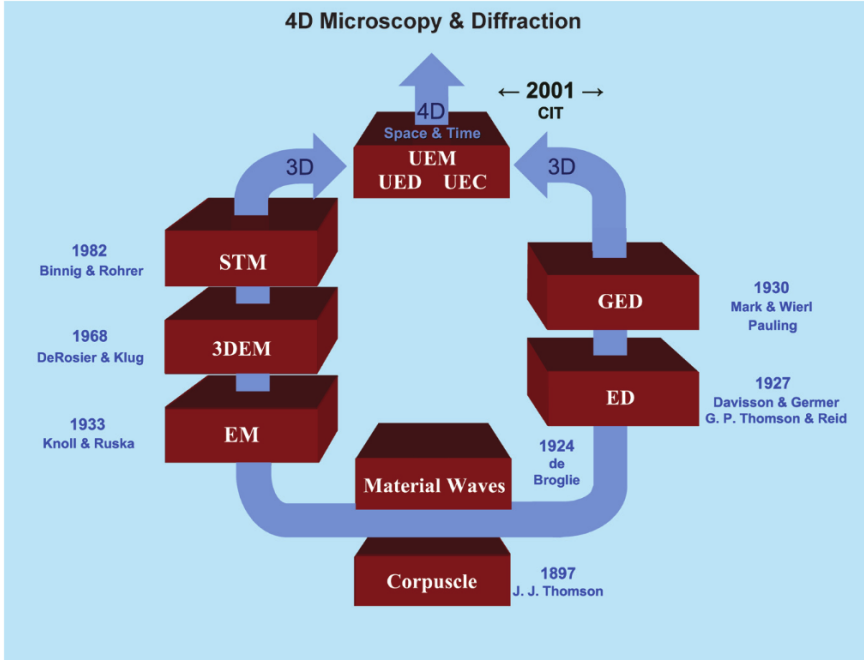

Fig. 11. Historical perspective with some milestones of developments in 2D and 3D imaging, in real and in Fourier space. The culmination in 4D microscopy and diffraction is shown in the confluence at the top.

5. Epilogue

At the end, it is the understanding of the nature of physical forces at different time and length scales, and the mechanism of function, which will provide the real meaning of emergence and complexity. In all of the above-mentioned applications, 4D electron imaging, in real or in Fourier space, is our method of choice at Caltech. As evident in many cases, e.g., those of chemical reactions, phase transitions, and nanoscale mechanical phenomena, both the spatial and the temporal resolution of imaging are essential for the trilogy construct of structure-dynamics-function. With time being the fourth dimension in 4D microscopy and diffraction, which have their historic roots in 2D and 3D methods (Fig. 11), we anticipate myriad of applications.

Uncovering the nature of biological complexity will surely benefit from the advances made in 4D visualization. Despite the enormous progress made so far, we are back to a 50-year-old physics question by Erwin Schrödinger — *What is life?* — but with a different perspective. In the coming 50 years, studies of the molecular basis of life in physical biology, together with the new approaches of systems and synthetic biology, will significantly advance our knowledge regarding what I believe to be some of the most important questions pertinent to biological complexity:

Life. How can a soup of chemical molecules form life? Does the origin of life involve a complexity of new paradigms beyond what we know now?
Humans. How does a network of organs result in a conscious being? How does our consciousness result in altruistic or destructive desires?
Organs. How does a network of ions, water, and proteins, for example, form the most powerful computing machine, the brain? How do we acquire, or lose, memory through the dense traffic of billions of neurons?
Cells. How does a "microfactory" with thousands of components attain dynamic stability? What is the origin of selectivity and fidelity in such a crowded environment, and what causes robust self-organization and self-assembly in noisy environments and nonequilibrium functional states?

Molecules. How do macromolecules fold or misfold uniquely and how do they recognize others in, for example, drug delivery and function? And, what makes a triatomic water molecule the most complex to understand and the most useful matrix of life?

These are "big questions" and the issues involved require knowledge of quantum and statistical physics, molecular bonding and synthesis, and network systems engineering and nonlinear information-flow dynamics. The hope is that the various powerful tools of molecular and systems biology, together with visualization, will make the science of biological complexity in the 21st century as revolutionary in its impact as was quantum mechanics in the 20th century.

The integration of physics and chemistry with biology is important, but it would be naive to think that the fundamentals of physics or chemistry as disciplines are at "the end of science," as claimed by some. Until today we still do not understand the real forces behind, for instance, dark energy and dark matter, nor do we understand why most of our universe is unknown, why the physical constants of nature are constant, why they take on their unique values, and what the purpose of duality, uncertainty, and chaos is. In chemistry, the nature of water in different phases, the forces behind self-assembly, and the recognition of molecules to others are frontiers that are still pregnant with major questions. Naturally, as we progress in acquiring new understanding, new questions will emerge. Lastly, because we cannot violate the second law, ordered biological phenomena, such as a tree, have to be considered in the context of universal interactions as the net entropy must increase. Thus, biological complexity, although local, it is part of our cosmic light-life realm, a concept recognized millennia ago (Fig. 12), and is appropriate for ending this piece.

Fig. 12. The significance of light-life interaction as perceived millennia ago, since Akhenaton and Nefertiti.

Acknowledgments

I wish to acknowledge the support of this research by the National Science Foundation, the Air Force Office of Scientific Research, and the Gordon and Betty Moore Foundation. Part of the biological imaging section represents the collaboration with Grant Jensen (NIH grant R01 GM081520-01) in the Physical Biology Center whose members are listed in Fig. 13. The dedicated effort by the group members who carried out the research discussed here on 4D visualization is acknowledged in the publications listed below. I would like to thank Dmitry Shorokhov for his care in formatting the text and for helpful discussion.

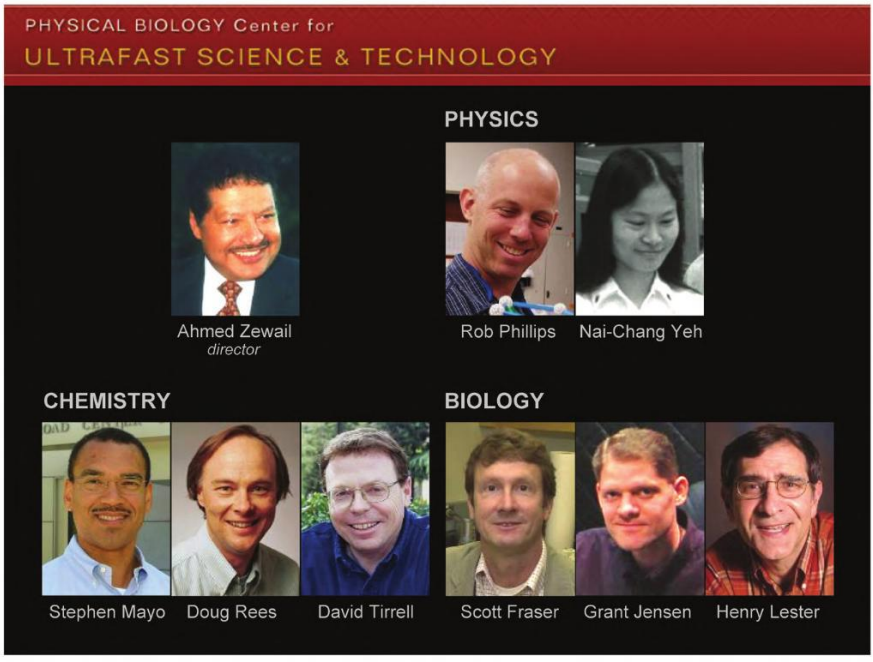

Fig. 13. Colleagues involved in the Physical Biology Center at Caltech.

Selected Publications

The following representative publications from Caltech are provided for readers interested in more details of the work on 4D visualization discussed in this chapter.

1. Zewail AH. (2008) Visualizing complexity: Development of 4D microscopy and diffraction for imaging in space and time. In: Chiao R, Phillips W, Leggett A, Cohen M, Ellis G, York D, Bishop R & Harper C (eds), *Visions of Discovery: Shedding New Light on Physics and Cosmology*, in press. Cambridge University Press, London.
2. Carbone F, Baum P, Rudolf P, Zewail AH. (2008) Structural preablation dynamics of graphite observed by ultrafast electron crystallography. *Phys Rev Lett* **100:** 035501.

3. Flannigan DJ, Lobastov VA, Zewail AH. (2007) Controlled nanoscale mechanical phenomena discovered with ultrafast electron microscopy. *Angew Chem Int Ed* **46:** 9206–9210.
4. Baum P, Zewail AH. (2007) Attosecond electron pulses for 4D diffraction and microscopy. *Proc Natl Acad Sci USA* **104:** 18409–18414.
5. Baum P, Yang D-S, Zewail AH. (2007) 4D Visualization of transitional structures in phase transformations by electron diffraction. *Science* **318:** 788–792.
6. Park HS, Baskin JS, Kwon O-H, Zewail AH. (2007) Atomic-scale imaging in real and energy space developed in ultrafast electron microscopy. *Nano Lett* **7:** 2545–2551.
7. Lobastov VA, Weissenrieder J, Tang J, Zewail AH. (2007) Ultrafast electron microscopy (UEM): Four-dimensional imaging and diffraction of nanostructures during phase transitions. *Nano Lett* **7:** 2552–2558.
8. Seidel MT, Chen S, Zewail AH. (2007) Ultrafast electron crystallography II. Surface adsorbates of crystalline fatty acids and phospholipids. *J Phys Chem C* **111:** 4920–4938.
9. Gedik N, Yang D-S, Logvenov G, Bozovic I, Zewail AH. (2007) Nonequilibrium phase transitions in cuprates observed by ultrafast electron crystallography. *Science* **316:** 425–429.
10. Zewail AH. (2006) 4D ultrafast electron diffraction, crystallography, and microscopy. *Annu Rev Phys Chem* **57:** 65–103.
11. Lin MM, Shorokhov D, Zewail AH. (2006) Helix-to-coil transitions in proteins: Helicity resonance in ultrafast electron diffraction. *Chem Phys Lett* **420:** 1–7.
12. Lobastov VA, Srinivasan R, Zewail AH. (2005) Four-dimensional ultrafast electron microscopy. *Proc Natl Acad Sci USA* **102:** 7069–7073.
13. Srinivasan R, Feenstra JS, Park ST, Xu S, Zewail AH. (2005) Dark structures in molecular radiationless transitions determined by ultrafast diffraction. *Science* **307:** 558–563.
14. Ruan C-Y, Lobastov VA, Vigliotti F, Chen S, Zewail AH. (2004) Ultrafast electron crystallography of interfacial water. *Science* **304:** 80–84.
15. Ihee H, Lobastov VA, Gomez UM, Goodson BM, Srinivasan R, Ruan C-Y, Zewail AH. (2001) Direct imaging of transient molecular structures with ultrafast diffraction. *Science* **291:** 458–462.

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Revolutionary Developments from Atomic to Extended Structural Imaging

*John Meurig Thomas**

Of the three kinds of primary beams (neutrons, X-rays and electrons) suitable for structural imaging, the most powerful are coherent electrons. The source brightness of such electrons significantly exceeds achievable values for neutrons and X-rays: their minimum probe diameter is as small as 0.1 nm, and their elastic mean-free path is *ca* 10 nm (for carbon), much less than for neutrons and X-rays. Moreover, because electrons are focusable and may be pulsed at sub-picosecond rates, and have appreciable inelastic cross-sections, electron microscopy (EM) yields information in four distinct ways: in real space, in reciprocal space, in energy space and in the time domain. Thus, besides structural imaging, energy landscapes of macromolecules may be explored, and under optimal conditions elemental compositions, valence states and 3D information (from tomography) may be retrieved by time-resolved EM.

Advances in designs of aberration-corrected high-resolution electron microscopes have greatly enhanced the quality of structural information pertaining to nanoparticle metals, binary semiconductors, ceramics and complex oxides. Moreover, electron tomography sheds light on the shape, size and composition of bimetallic catalysts attached to nanoporous supports. With energy-filtered electron tomorgraphy, chemical compositions of sub-attogram quantities located at the interior of microscopic objects may be retrieved non-destructively.

Radiation damage is the main problem which prevents the determination of the structure of single biological macromolecules at atomic resolution using any kind of microscopy, irrespective of the primary beam. Great advances have recently been made via the EM of biological macromolecules and machines embedded in vitreous ice. Electron cryomicroscopy is now used to analyze the structures of molecules arranged as crystals or as single particles; and cryoelectron tomography reveals nuclear pore complex structure and dynamics.

*Department of Materials Science, University of Cambridge, Cambridge, CB2 3QZ, UK.

Electron energy loss spectroscopy and imaging, especially energy-filtered (S)TEM tomography is an important development in the imaging of both inorganic and biological systems; and Zewail's revolutionary single-electron (4D) ultrafast-EM and diffraction contribute richly to the structural imaging of both atomic and macromolecular species at sub-picosecond time scales.

1. Introduction

If our aim is to determine and visualize, at near-atomic or sub-nanometer resolution, the structure, architecture and general architectonic features of various macromolecular or cellular or extended inorganic materials, few instruments currently surpass, in the panoply of powerful procedures that it offers, the modern high-resolution electron microscope. This chapter, therefore, deals predominantly, but not exclusively, with the primacy of electron microscopy in its various manifestations and the several ways in which it reveals information that interests us.

Visualization alone is not enough. To achieve maximum insight from any worthwhile means of imaging, one should — for relatively simple or quite complicated inorganic systems — also learn about elemental composition, degree of structural flexibility, valence states and modes of bonding of the constituent atoms. And for more complicated biomolecular and cellular entities, the functional role of (or mechanistic details associated with) the entities investigated need, ideally, to be revealed by the particular technique of imaging that one adopts. To be specific, in the act of visualizing the double-stranded (ds) DNA genome in say, a bacteriophage, one needs to know not only the compact arrangement of the DNA but also how it is topologically organized to facilitate efficient ds DNA release during infection.

It is instructive at the outset to recall that perfectly reliable experimental methods already exist for imaging the two extreme kinds of structures that define the continuum of materials from atoms, on the one hand, to living cells, on the other. Intermittently throughout this chapter, we shall cite a number of revolutionary examples from the inorganic world, where the limitations that arise from electron-beam damage are generally small.