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# The scientific legacy of Martin Karplus from the perspective of his collaborators

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ABSTRACT The work of Martin Karplus, who passed away on Dec 28, 2024, was at the forefront of computational chemistry and molecular biophysics for a period of more than sixty years. His career started with a PhD in theoretical chemistry at Caltech with Linus Pauling in 1953. After performing leading research in molecular quantum chemistry for two decades, in the 1970s, he began to incorporate work on biological systems, and his work was instrumental in creating modern computational molecular biophysics. In 2013, he was awarded the Nobel Prize together with Arieh Warshel and Michael Levitt "for the development of multiscale models for complex chemical systems." This article aims at briefly reviewing the

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SIGNIFICANCE Martin Karplus, who passed away on Dec 28, 2024, was at the forefront of computational chemistry and molecular biophysics for more than sixty years. In 2013, he was awarded the Nobel Prize in Chemistry with Arieh Warshel and Michael Levitt "for the development of multiscale models for complex chemical systems." The main achievements of the Karplus lab are illustrated from the point of view of the people working under his unique mentorship.

main achievements of the research performed in the Karplus lab from the point of view of the people working under his unique mentorship.

## INTRODUCTION

The work of Martin Karplus, who passed away on December 28, 2024, was at the forefront of computational chemistry and molecular biophysics for six decades (Fig. 1). His career started out with a PhD in theoretical chemistry at Caltech with Linus Pauling in 1953 entitled "A quantum-mechanical discussion of the bifluoride ion." Karplus then became a leader in theoretical chemistry for over two decades before beginning to incorporate work on biological systems at atomic detail in the early 1970s. In 2006, he wrote a review entitled "Spinach on the Ceiling," which described his viewpoint of his long career (1), followed by a more complete account in his biography (2). In 2013, he was awarded the Nobel Prize with Arieh Warshel and Michael Levitt "for the development of multiscale models for complex chemical systems" (3). This article discusses research done in the Karplus lab from the viewpoint of some of his co-workers, and to set the scene, it is worthwhile to briefly summarize the contour of the main achievements of his career.

Martin Karplus began his faculty career in the Department of Chemistry at the University of Illinois at Urbana–Champaign in 1955. This department was already playing a central role in advancing nuclear magnetic resonance (NMR) applications to chemistry with pioneers like Herbert Gutowsky and Charles Slichter. Karplus was also inspired by Raymond Lemieux's lecture on sugar conformations and his measurements of so-called vicinal J-couplings. Lemieux's observations matched Karplus's ideas, motivating the formulation of the Karplus equation (4,5). In 1964–65 and then at Columbia's IBM Watson Laboratory and Chemistry Department, Karplus made major advances in studying the $H + H_2 \rightarrow H_2 + H$ reaction. Working with Richard Porter and Ramesh Sharma, he computed a Born-Oppenheimer potential energy surface and performed the first quasiclassical scattering studies of this three-body system (6,7). This work showed that the reaction could be treated using classical nuclear motion on an electronic potential surface. Although the early surfaces lacked full accuracy, they enabled meaningful comparison with experiments. This trajectory ultimately inspired Karplus to extend classical simulations to proteins using empirical potentials. Karplus moved to Harvard in 1966. In 1969, he spent a sabbatical visit at the Weizmann Institute, where he visited chemical physicist Shneior Lifson, who at the time was working on the development of empirical potential energy functions to describe the energy surfaces of polymers and small molecules. Lifson's group was a leader in the use of such "force fields" for complex molecules, including the development by Lifson and Warshel of the so-called consistent force field (CFF) (8,9). This force field directly included van der Waals interactions together with electrostatic interactions between the partial charges attributed to atoms that are not directly bonded covalently, which was an innovation at the time.

Returning to Harvard after the sabbatical visit, Karplus wished to work on a biologically relevant problem. Although lacking formal biological training, he turned to retinal, a system he knew from earlier work with Hubbard and Wald. Retinal, a polyene chromophore central to vision, especially its 11-*cis* isomer, undergoes photoisomerization when bound to opsin to form rhodopsin. Karplus saw retinal as an ideal candidate for semiempirical CFF methods. With Barry Honig, he calculated ground-state potentials and proposed a 3D structure of the chromophore (10). Also, collaborating with Warshel, he extended CFF approaches to conjugated molecules and later developed semiclassical trajectory methods to model photoisomerization and excited-state dynamics (11). This line of investigation, which pioneered the so-called quantum mechanics/molecular mechanics (QM/MM) approach, was later expanded in the PhD work of Klaus Schulten (12,13). The focus of the Karplus lab shifted more resolutely toward biological macromolecules in the early 1970s, when graduate student Attila Szabo developed a discrete-state statistical mechanical model to explain cooperative oxygen binding in hemoglobin (14). They constructed a simplified model built from a finite set of functional states, foreshadowing the now well-established mappings of protein conformations onto metastable states using data from molecular dynamics (MD) simulations (15). By the end of this work, Karplus realized that answering the next round of questions raised by this model required calculating hemoglobin's energy as a function of atomic positions. Graduate student Bruce Gelin became central to this effort. To connect the statistical model's effective free energy states with an atomistic representation, Gelin worked to develop methods for computing energies from atomic coordinates, with guidance from Karplus and drawing on Lifson's theoretical framework and Warshel's CFF program that was made available to Karplus. In

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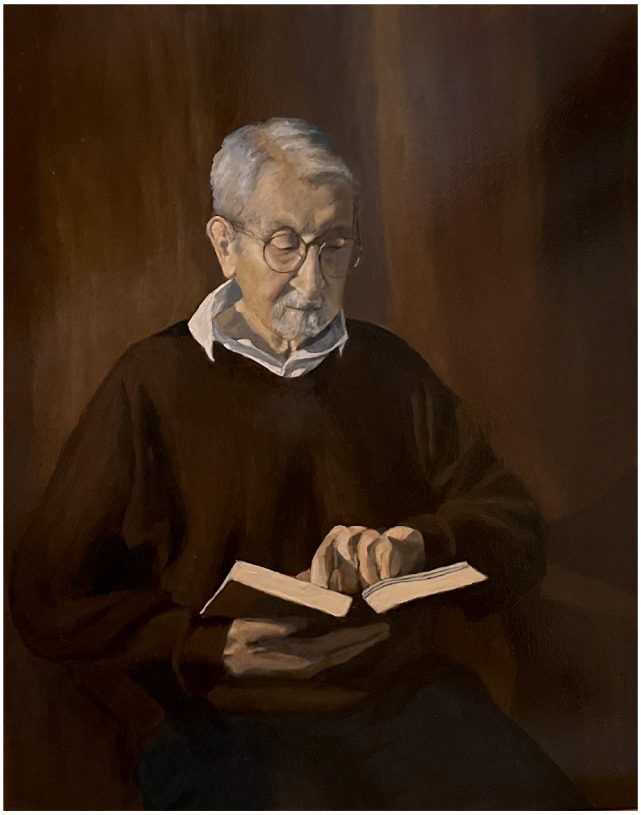

FIGURE 1 Martin Karplus (Spring of 2024). Oil painting by Marci Karplus, done in the style of Dutch portraits.

1975, Gelin and Karplus published a pioneering study on amino-acid side-chain motion in bovine pancreatic trypsin inhibitor (BPTI) (16). Then followed a study examining the hinge-bending mode in lysozyme (17) (a precursor of quasi-harmonic models). Building on Gelin's force calculations, McCammon implemented an integration scheme capable of computing atomic trajectories. This breakthrough enabled MD simulation of the internal motion and conformational fluctuations of a small protein BPTI (18), laying groundwork for modern all-atom MD simulations of biomolecules. It is during this period that the molecular mechanics computer program CHARMM started to be systematically designed (19–21). Suddenly, the ability to carry out detailed atomic-level MD simulations of proteins made it possible to perform sophisticated statistical mechanics on these exceedingly complex systems (22). Even after fifty years, modern computational biophysics is still living in the aftermath of this major landmark period.

Martin Karplus had a very large number of lab members, who affectionately refer to themselves as "Karplusians." There are more than 250 Karplusians, including all graduate students and postdocs. In the following, a number of them complement the memoirs written by Karplus himself by briefly summarizing the advances that they made under Martin's mentorship as well as the impact of their work as seen in hindsight.

## CONTRIBUTIONS

### Molecular dynamics

In the early 1960s, Martin Karplus studied reactive collision in the gas phase, focusing on the simple $H + H_2$ reaction. An important landmark was to introduce the quasiclassical trajectory (QCT) method to the field of chemical reaction dynamics (6,7). Building on earlier work (23,24), the 1964/1965 publications present results from dynamics studies "without restrictive approximations." Characteristically for Martin's style, the 1965 legacy paper ends with "Cautions," emphasizing the crucial role of accurate potential energy surfaces for obtaining physically meaningful trajectory simulations. These pioneering studies laid the foundation for subsequent work in which the nuclear dynamics of reactive systems were treated at a fully quantum-mechanical level (25), largely confirming the validity of the first QCT results for the benchmark $H + H_2$ exchange reaction. The results showed that relying on classical trajectories of atomic and molecular systems, while being an approximation, could yield meaningful insight (26). Given the long-time interest of Martin Karplus for biology, these conceptual advances progressively led to the use of classical trajectories for studying the dynamics of larger molecules such as proteins (27).

#### *The first MD simulation of a protein*

In 1972, Andy McCammon returned to the chemical physics PhD program at Harvard after two years of alternate service at Massachusetts General Hospital. At MGH, he conducted radioimmunoassays of hormones and became interested in protein motions when evidence arose for cooperative binding effects in some of the antibody molecules used (28). Could hinge-bending allow the binding domains to cooperate? Andy hoped to work with Martin for his Ph.D., but, due to Martin moving to France, he arranged for day-to-day supervision by John Deutch of MIT, so was accidentally trained in statistical mechanics and hydrodynamics. He then started a postdoc with Martin in 1976. Interest in protein dynamics was stirring, in part due to NMR studies of hen egg lysozyme (29) and the small protein, BPTI (30,31). Martin suggested that Andy try to think of how motions in a protein might be modeled on a computer. In browsing Lehninger's biochemistry textbook the next day, Andy was struck by an image of lysozyme, with two globular lobes connected by a hinge-like segment. This reminded him of the globular lobes and hinges of antibody molecules, and he wondered if one could model the hinge-bending motion. Martin was very encouraging. Using Bruce Gelin's elegant software for protein molecular mechanics, they computed the energetic cost of bending one lobe toward the other, around the hinge axis, with partial energy minimization to reflect the relaxation of the protein during bending. With the resulting approximately quadratic potential for bending, and simple models for hydrodynamic damping, equations of motion for the

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harmonic motion were developed, indicating an overdamped, diffusional opening and closing of the active site between the lobes with a decay time of about 20 ps. This work was published in *Nature* in 1976 (17). Several years later, some of these ideas were expanded by Ron Levy to develop quasi-harmonic models (32–34).

Attention rapidly turned to a more comprehensive study of protein dynamics, and the recent experimental studies of the small BPTI molecule made this protein an excellent candidate. A CECAM workshop on protein dynamics had been organized in Orsay, France, by Prof. Herman Berendsen, and Martin arranged for Andy to attend. The workshop had access to generous amounts of time on an IBM mainframe computer. Anees Rahman kindly supplied the MD module from software that he had developed for simulations of liquids, and Andy combined this with Bruce Gelin's molecular mechanics software to create a protein MD package. After some amusing misstarts (35), a simulation of BPTI dynamics for 9 ps was completed and analyzed (18). Key findings of this study, e.g., larger amplitude motions on the surface of the protein, "liquid-like" aspects of the internal motion, and correlated displacements due to close packing, have been observed in subsequent protein MD simulations and experimentally. The observation that the interior of protein displayed a liquid-like behavior returned a number of times in the following decades (involving ion channels, Lindemann criteria, etc.). Martin and Andy collaborated in many subsequent protein MD studies, including studies of activated processes such as the rotation of the tyrosine rings that had helped to arouse interest in protein dynamics (36,37). That work led to the first presentation of a multidimensional reaction energy surface for a dynamical transition in a protein (36). Later, Elber and Karplus would provide the first estimate of the global ruggedness of a protein energy landscape including an estimate of the rates of transition between energy minima (38).

### *Allostery and cooperativity: Hemoglobin and myoglobin*

One of the enduring features of Martin's scientific career was an interest in heme proteins, especially hemoglobin and myoglobin, which bind oxygen in red blood cells and in muscles. This interest was driven by early X-ray structures, and by ground-breaking kinetic studies by the Frauenfelder group (39). This led to a long-term interest in the subject of allostery and cooperativity, which touched many Karplusians. In the early 1970s, hemoglobin, the larger of the two proteins, with four polypeptide chains and two main quaternary structures, was the focus of much attention. Work with Attila Szabo, including one famously acknowledging a Parisian cafe, Les Deux Magots (14), created statistical framework models for the binding of both oxygen and other ligands to hemoglobin, with states contributing to the ensembles inspired by structural features from X-ray work (14). It is noteworthy that this work on allostery in hemoglobin made it clear that the next step would be to find ways to calculate the energy of different protein conformations, leading ultimately to the adoption of atomic force fields and MD simulations (27).

Later, Dave Case used force fields and MD to describe fluctuations in myoglobin (the smaller of the two proteins) that allowed small molecules to bind to a buried iron atom in the interior of the protein (40). Bruce Gelin and Angel Lee (41) described structural aspects of a pathway between quaternary structures. The following decade saw what was, at the time, a very long simulation (of 300 ps duration) of myoglobin (42), which led to a flurry of studies of the dynamic behavior of myoglobin, both in solution (38,43–49) and in crystals (50–53). This included time-resolved studies of ligand dissociation and energy transfer by Philip Anfinrud and then junior faculty at Harvard University, in which observed timescales were found to be in quantitative agreement with MD simulations (54).

The advent alchemical free energy methodologies like thermodynamic integration (TI) and free energy perturbation (FEP) in the mid-1980s led to a study of the "hidden thermodynamics" of mutations in hemoglobin by Jiali Gao, Bruce Tidor, and Krzysztof Kuczera (55). Jiali Gao joined Martin's group as a postdoc in December 1987, right before Martin's sabbatical leave in France. This was a somewhat unusual start to a postdoc. Having carried out FEP simulations using Monte Carlo simulations at Purdue with Bill Jorgensen, Jiali and Krzysztof collaborated and started free energy simulations on one of the key hemoglobin mutations (Asp$\alpha$99Ala) at the interface associated with the conformational transition between oxy and deoxy states and on the Glu$\beta$6Val mutation that causes sickle-cell anemia—the first "molecular disease," as named by Pauling (55). Bruce Tidor had written a module in CHARMM to partition a system into different blocks, which could be used to create a weighted hybrid energy function.

Coming from a background in physics and physical chemistry of gas-phase systems, Markus Meuwly joined the group in fall of 2001. The original aim of the project was to use what would nowadays be called "machine-learning-based energy functions" in MD simulations. As it so happens, the first two projects he worked on did not have anything to do with this, but rather concerned the dynamics of the NO-rebinding reaction to myoglobin (MbNO) and proton transfer reactions (56,57). During his postdoc time with Martin, none of the maching-learning-based techniques were pursued; this had to wait until the late 2010s (58). The MbNO project had been started in 1992 by Oren Becker but was unfinished. This was an ideal point of departure for Markus to become familiar with the technique of MD. The common interest in heme-containing proteins culminated in a study that attempted to understand the inability of molecular simulations to correctly rank the two conformational substates of tetrameric hemoglobin: T- versus R-Hb. Again, this work was a paradigm for Martin's

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approach to science: his absolute belief in the power of a high-quality molecular simulation paired with an encyclopedic knowledge of the literature and the mastery to apply these tools to address a fundamental problem (59). As was also often the case, such studies were sufficiently controversial to make fellow scientists rethink their beliefs.

Antoine Taly joined the Karplus group in Strasbourg in 2005 after a seminar presenting the study of the nicotinic acetylcholine receptor, an allosteric protein studied with Jean-Pierre Changeux at the Pasteur Institute (60,61). The activation mechanism had been modeled with a coarse-grained normal mode analysis, and the discussion, initiated by Roland Stote, quickly revolved around the opportunity to model the effect of ligands in the framework of this representation. Playing with the granularity became the first subject of Taly's postdoc, showing that allosteric sites were remarkable in terms of their potential to alter functionally relevant normal modes (62). The subject was later expanded by Marco Cecchini using MD allowing combination of the twisting motion identified by with more local rearrangements (63).

Martin's interest in allostery continued over the years, with collaboration with Karplusians long after they had left the group. For example, Qiang Cui had been a postdoc during 1997–2000. Once he shared his group's work on the analysis of allostery in a single domain protein (64,65), Martin suggested to write a review together to critically discuss the "old" and "new" views of protein allostery (66). That article (67) helped (re)clarify several fundamental concepts concerning cooperativity in proteins, which remained a topic of interest to Qiang to this day (68).

### *Protein folding*

Protein folding is one of most enduring problems of computational biophysics. The relation between the sequence of amino acids of a protein and its three-dimensional folded structure has fascinated scientists for decades. Martin was more interested in the physical aspects of the folding mechanism itself, rather than the prediction of the folded structure given a sequence. For instance, the diffusion-collision model was developed with David Weaver (69,70) in part to address the fundamental questions raised by Levinthal on the kinetics of folding (71). This type of work on protein folding continued during all of Martin's career.

Aaron Dinner joined the Karplus group as an undergraduate student in 1992, initially working closely with Andrej Šali and Eugene Shakhnovich, who had recently moved into an independent position, on studies of lattice models of protein folding. After they explored exhaustive enumeration of conformations of short chains (72), Martin asked Aaron to look into long chains that could support secondary structures (73), with a view toward connecting with the diffusion-collision model that Martin had developed with David Weaver (69,70). The advantage of the lattice models, in Martin's words, was that one could "just watch them fold." The studies of the long chains carried over into Aaron's PhD. Motivated by Martin's work with Sung-Sau So on quantitative structure-activity relationships (74,75), they incorporated machine learning methods (76) that would, in later stages of Aaron's career, prove useful for identifying factors determining experimental folding rates (77) and committor functions (78)—probabilities of reaching product states before reactant states. Following a revival of interest in committor functions (79), a study correlating lattice model structures with thermodynamics and kinetics of folding established a template for studying complex dynamics (80). This template would be revisited as it became possible to use all-atom models to study protein folding (81), DNA repair (82), and insulin dimer dynamics (83), all topics on which Martin left his mark (84–89). Because the lattice models relied on Monte Carlo methods, these studies also prompted a reexamination of whether Monte Carlo methods could be useful for studying all-atom models. The resulting Monte Carlo module in CHARMM (90–92), together with an implicit solvent model that Themis Lazaridis developed with Martin (93), enabled one of the first all-atom studies of $\beta$ hairpin formation (94).

Yaoqi Zhou joined Martin's group in September 1995 as an NSF- and NIH-funded postdoctoral fellow. Trained in the statistical mechanics of simple liquids during his PhD at Stony Brook University, he later learned discontinuous MD of homopolymer liquids (95) during a brief postdoctoral stint with Carol Hall at North Carolina State University. He entered Karplus's group with no prior experience in biological systems. In Karplus's lab, Dr. Zhou was fascinated by learning that isolated protein chains could spontaneously fold into their functional structures. This inspired him to investigate isolated homopolymer chains, leading to the unexpected observation that even a homopolymer exhibits three phase transitions analogous to bulk matter: gas-liquid, liquid-solid, and solid-solid polymorphic transitions (96,97). Notably, the liquid-to-solid transition of a single chain was identified as a first-order, two-state disorder-to-order transition—a finding with implications for understanding protein folding landscapes. A critical lesson from this work, shaped by Karplus's mentorship, was the importance of precision in scientific definition. Initially, Dr. Zhou utilized an approximate metric (inter-particle distance fluctuations) to characterize the solid phase. Karplus, however, emphasized that the Lindemann criterion (98), which determines the liquid nature of a melting solid from the fluctuations around equilibrium positions, was the physically rigorous standard. Indeed, correcting this seemingly trivial difference yielded a more accurate description of the solid phase, reinforcing the value of methodological rigor (99). This principle became a cornerstone of Dr. Zhou's research philosophy, later distilled into his essay circulated on the internet: "Recipe for a Quality Scientific Paper: Fulfilling Readers' and Reviewers' Expectations" (100). Expanding

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on the insights learned from homopolymer transitions, Dr. Zhou adapted square-well homopolymer models to study proteins. The computational efficiency of these models enabled, for the first time, full thermodynamic and kinetic analyses of an off-lattice model for protein folding. His simulations revealed that native proteins adopt a surface-molten solid state (101), a conclusion later validated by all-atom simulations of ribonuclease A, barnase, myoglobin, and crambin (102). Further work demonstrated that helical proteins could fold via very distinct mechanisms (diffusion-collision or collapse-reorganization), culminating in a *Nature* paper on folding kinetics (103).

### *Lipid membranes*

Richard Pastor joined the group as a graduate student in 1978 and decided to work on membranes, rather than proteins. Simulating a membrane in the early 1980s was no small thing. Other groups had simulated chain models (104), and soap bilayers (105), but the science and technology for an adequately sized phospholipid bilayer were not yet available. This is where Martin's approach to science became critical. Pastor attended a Gordon Conference in 1981 and heard a talk by Michael Brown. Brown argued that the nanosecond relaxation time observed in his frequency-dependent NMR measurements of lipid bilayers arose from collective motions of many lipids (106). Upon returning from the meeting, Pastor proposed to Martin that he use Brownian dynamics to determine whether a model of uncorrelated lipid rotation (sometimes called wobble in a cone) from Bocian and Chan (107) could fit Brown's results. Martin agreed. In order to limit the number of particles in the simulation, the simulation model consisted of a single lipid chain in a restraining potential. Pastor had a string of failures, as none of the obvious choices, like a conical potential, resulted in the experimental pattern of chain deuterium order parameters. Then he came across a paper by Schindler and Seelig (108) who used a mean field model to generate order parameters. He told Martin that this looked promising, but he was not sure what to do next. Martin agreed to read the paper. The next morning, he simply said, "It's a potential of mean force." That was all Pastor needed. His simulations worked, he got his PhD, and he built on this study for many years (109). In fact, the CHARMM36 lipid force field (110) is partially based on insights gained from Pastor's work with Martin.

### *Ion channels*

Benoît Roux was admitted as a graduate student to the Biophysics PhD program in 1984, coming from the physics department at the University of Montreal where biophysics was highly focused on membrane transport. Martin assigned Benoît to a collaborative project with Professor David Busath from Brown University on the gramicidin A channel. At the time, this project was being carried out by Kevin Gaffney who was a chemistry undergraduate. This was exciting. Just before coming to Harvard, Benoît had been very impressed by a presentation by Professor Kent Wilson from UCSD at a Biophysical Discussion organized by Adrian Parsegian of the first atomically detailed MD simulation of a molecular pore, the gramicidin A channel (111). Yet, the ion channels community at the time primarily lived within a world of kinetic models based on Eyring rate theory (112). Aiming for simplicity, Benoît decided to begin with an infinite periodic gramicidin-like helix. He determined the potential of mean force (PMF) of $Na^+$ and $K^+$ ions in this system (113) and calculated the transition rates using the reactive flux formalism (114), a method that Martin and Andy McCammon had used to characterize the tyrosine ring flip in BPTI a few years earlier (37). Benoît's PhD thesis culminated with the calculation of the PMF of $Na^+$ along the axis of the head-to-head right-handed gramicidin A dimer channel based on the NMR structure by Arseniev et al. (115). Over a period of a few weeks during the holiday season (1989–1990), the PMF calculation exhausted the entire annual allocation of the Karplus lab on the Cray Y-MP at the Pittsburgh Supercomputer Center. The manuscript, which was rejected by several journals, ended up being published in 1993 a few years after Benoît's graduation (June 1990) (116). While the free energy barriers were too high and inconsistent with the measured channel conductance, a striking result was the prediction of the deep free energy minima along the pore axis, around $\pm 9.5$ Å from the center of the dimer. Experimental measurements later independently confirmed the positions of the main ion binding sites in the gramicidin A channel (117,118).

Despite the limitations of classical force fields, it appeared that rigorous PMF calculations with enhanced sampling strategies could indeed accurately predict the position of ion binding sites along a narrow molecule pore. Perhaps, then, this type of calculation could be useful to understand the function of more complex biological channels. However, it would still take several years to test this idea. Finally, in 1998 the X-ray structure of the KcsA $K^+$ channel from *Streptomyces lividans* was determined by Doyle et al. (119). Adding a new complication compared to the gramicidin channel is the fact that $K^+$ channels are multi-ion pores. After failed attempts to carry out a PMF calculation along some effective one-dimensional reaction coordinate, Simon Berneche, who was a graduate student, took it upon himself to expand the PMF calculation to two dimensions with more than 300 umbrella sampling windows. With the aid of the weighted histogram analysis method (WHAM) (120), the abundant information from all these biased simulations could be recombined fruitfully to yield a first multi-ion PMF for a $K^+$ channel (121). At 3.2-Å resolution, the 1998 KcsA X-ray structure displayed possibly two $K^+$ ions bound in a narrow selectivity filter. The PMF calculation revealed that there were five cation binding sites along the selectivity filter, called $S_0$ to $S_4$

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from the extracellular side to the intracellular side. At the same time, and unknown to Benoit, the lab of Rod MacKinnon obtained a new structure at 2.0-Å resolution, showing the same binding sites (122). This remarkable prediction gave confidence to rely on MD simulations to understand one important aspect of these channels, namely their high selectivity that makes them favor $K^+$ over $Na^+$ ions. Alchemical FEP calculation showed that, indeed, the KcsA channel is selective for $K^+$ over $Na^+$ (123). However, while selectivity is commonly attributed to a structurally precise fit between the $K^+$ ion and a rigid pore, the simulations showed that the carbonyl groups coordinating the ion were indeed very dynamic. Selectivity for $K^+$ emerges as a robust feature of a flexible pore with fluctuating carbonyl ligands acting as a confined "liquid-like" droplet (124), recalling some of the earlier observations from the first MD simulation of a protein (18).

### *Nucleic acids*

In March of 1983, Lennart Nilsson and wife Anna came from Stockholm to the Karplus group at Harvard, on a stipend to Lennart from the newly established postdoctoral program of the Swedish Natural Sciences Research Council. Lennart had just defended his PhD thesis involving Monte Carlo simulations of the effect of mRNA secondary structure on ribosome movement along mRNA, as well as SAXS and time-resolved fluorescence studies of nucleic acids, primarily tRNA. The main purpose of Lennart's postdoc with Martin Karplus was to learn the new MD simulation methodology and use it to try to understand some of these experimental results, which indicated that there were two to three different conformational states of the tRNA in a dynamical equilibrium. At the time, however, MD simulations of nucleic acids were not entirely successful: the high surface charge density of nucleic acids due to the negatively charged phosphates in the backbone of DNA and RNA was significantly more challenging than the proteins that had so far been simulated *in vacuo*. Different approaches, such as reduction of the phosphate charges or addition of hydrated counterions, had been applied to fix this shortcoming and to maintain stable DNA structures in the simulations, but clearly these were ad hoc remedies, which did not properly address the underlying problem of the strong electrostatic interactions. Lennart was first assigned the task of cleaning up the CHARMM force field for nucleic acids, in particular to optimize the parameters for the internal coordinates, where there were some issues regarding computed vibrational spectral lines in the range 2,000–3,000 $cm^{-1}$, which should be empty. With this accomplished in 1984 (125), Martin one day asked Lennart to look at some experimental data obtained by Marius Clore and Angela Gronenborn for a short DNA duplex, to see if this information could be used to determine the three-dimensional structure of the DNA duplex. These were interproton distances from nuclear Overhauser enhancement (NOE) experiments, which Lennart could easily introduce as restraints in MD simulations using the flexibility of the restraint functionality available in the CHARMM program, without adding any new code to the program. Using MD simulations (only 8 ps!) at elevated temperatures, Lennart showed that with the aid of the experimental data, simulations started either from an A-conformation, or from a B-conformation, converged to what was essentially a B-conformation (126), something that was not feasible using energy minimization techniques. A decade later, available computer power had increased sufficiently to allow MD simulations of nucleic acids in a more realistic environment, including explicit water molecules and counterions, which essentially resolved earlier problems. Today, MD simulations of nucleic acids have become abundant. Much of this has depended on accurate parametrization of the nucleic acid force fields, in CHARMM as well as in AMBER and other programs. Lennart's two postdoctoral years (1983–1984) with Martin were formative for his further research; particularly important was the strong emphasis on the importance of interaction between computation and experiment, whenever possible. For nucleic acids, this included, among other things, the glass transition in DNA, occurring at around 230 K, roughly similar to what had been seen experimentally, and subsequently also shown computationally, for proteins. The main cause of the transition could be attributed to changes in the dynamics of the water environment (127), hence the similarity to the glass transition seen for proteins. The stacking properties of DNA and RNA dinucleotides were also comprehensively investigated using PMF calculations at about the same time (128). Later on, further investigations of various nucleic acid structural and chemical variants followed (triple helices, LNA, PNA, …), as well as studies of protein-nucleic acid complexes. The initial goal of Lennart's postdoctoral period with Martin, to study the dynamics of tRNA, was not really met until 30 years later, when a comprehensive parametrization of naturally occurring modifications in RNA had been achieved (129,130).

### *Enzymes*

The fundamental processes and principles underlying enzyme catalytic activity were another theme of Martin's work and fascination with biology (131). As he noted (132), the problem of enzyme catalysis can only be solved when an enzyme reaction can be described in the level of atomic detail as his work had revealed earlier for simple reactions such as $H + H_2 \rightarrow H_2 + H$. He showed that molecular simulations are essential for obtaining the necessary level of insight. This includes understanding of the processes of chemical bond breaking and bond making within enzymes, and also of protein dynamics and the conformational changes that are an integral part of enzyme reaction cycles. He developed methods capable of modeling chemical reactions within enzymes, in particular, multiscale

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combined QM/MM methods (see multiscale QM/MM simulations below), which have proved widely useful and for which he, Levitt, and Warshel were awarded the 2013 Nobel Prize in chemistry. He developed practical methods for modeling reaction paths in large systems such as enzymes and also showed how MD simulations could be used to reveal and understand the functional significance of the conformational changes in enzyme-catalyzed reactions. An example of both is his work on the prototypical enzyme triosephosphate isomerase (TIM) (133). His pioneering QM/MM investigation of this enzyme-catalyzed reaction revealed the catalytic role of a neutral histidine residue and the importance of its neutrality for efficient catalytic turnover(134). With Qiang Cui, he later investigated the role of quantum tunneling and other factors in the TIM reaction (135). As Martin remarked in his Nobel Lecture, enzymes such as TIM have been described as "perfect," in that their chemical reactions may be so fast that the overall reaction rate is limited by conformational changes. This, and his fruitful collaboration with the experimental work of Greg Petsko, motivated his MD simulations of active site conformational changes in TIM with Diane Joseph-McCarthy and others (section drug design below).

## Computational methodologies

### *The CHARMM program*

The original code used for the first MD simulation of a protein (18) was tailored specifically to BPTI. All atom arrays had fixed dimensions for BPTI's heavy atoms and polar hydrogens. Special routines added the disulfide links, again written only for BPTI. Paul Weiner had taken this assortment of programs to UCSF in 1978 as the basis of what became the AMBER package, which today remains a collection of multiple programs. By late 1979, the different codes had been merged into a single script-driven program, largely through the efforts of Bob Bruccoleri, with contributions from Olafson and Swaminathan. However, this program, which is sometimes referred to as "pre-CHARMM" (1,2), was in poor shape. Its structure consisted of three major sections: (1) the merged routines inherited from Gelin's BPTI-specific code; (2) Bruccoleri's structured analysis facility written in FLECS, a macro-based extension of FORTRAN that Bruccoleri promoted because it enabled more elaborate control structures; and (3) a third block of routines responsible for copying data structures back and forth between the other two, functioning as a defensive buffer. Documentation was minimal—only one test case and one page of instructions. Roughly half the code was in standard FORTRAN and the other half in FLECS. Karplus preferred not to intervene in programming-language decisions, and the postdocs often disagreed about the best approach, but Bruccoleri eventually prevailed, and the entire codebase was converted to FLECS. Even the name of the program had not yet been decided, with Gandalf and HARMM being considered, finally settling on the name CHARMM in the lead up to the 1983 paper (19).

Bernie Brooks came to the Karplus lab as an NSF Postdoctoral Fellow in 1980 with a strong programming experience from the group of Henry F. Schaefer III at UT Austin, where he had developed several novel quantum chemistry methods, including the first analytic gradients for correlated wavefunctions (Schaefer had just moved from UC Berkeley to UT Austin where Peter Rossky had just started as assistant professor). Bruccoleri's FLECS code was elegant, but its structure later proved incompatible with modern FORTRAN standards. The rest of the code lacked consistency and reliability (for example, an erroneous PATCH code for a BPTI simulation deleted chemical bonds, causing separate pieces of the protein to float away in space). Bernie's first major task in 1980 was the creation of the normal-mode facility VIBRAN. He soon moved on to developing the CORMAN and extending INTCOR modules and the CORREL facility. He redesigned the PSF-generation process by introducing the PATCH command, eliminating the need for system-specific patching routines. Bernie also developed the leapfrog integrator for MD, which remains the default algorithm. During this period, he worked on all aspects of the program except Bruccoleri's analysis facility, improving data structures and coding standards. By the time the first CHARMM paper appeared in 1983 (19), the group had assembled two dozen reliable test cases. Bernie, the first author of that paper, was responsible for the majority of the program by then. One of Bernie's most important contributions beginning in 1980 was the development of robust tools for macromolecular normal-mode analysis (136). He wrote the VIBRAN module early in 1980, spurred largely by Ron Levy's needs for better analysis capabilities. Several features, such as the PED command, were implemented at Levy's suggestion. VIBRAN quickly grew to 5,000 lines of code for Hessian construction, manipulation, and analysis. An additional 2,000 lines of second-derivative code were added elsewhere in CHARMM, including in the energy routines and Newton-Raphson minimizer. These capabilities supported numerous studies of large biomolecular systems, including the analysis of hinge-bending motion in lysozyme (137). Much of this work was not fully published until 1995 (138). In 1983, Bernie wrote the IMAGE facility, giving CHARMM general symmetry and periodicity capabilities. This approach, based on image atoms, allowed simulations of "infinite" $\alpha$ helices, $\beta$ sheets, and DNA, aiding parameter development. Early crystal simulations of cyclo-hexapeptides were particularly important in the development of PARAM19. Bernie also introduced numerous restraint types and expanded command parsing in collaboration with Axel Brunger. By the time he left in 1985, Bernie had written more than half of all CHARMM code. The first formal publication describing CHARMM appeared in 1983 (19);

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only two additional major publications have followed (20,21). Bernie left the Karplus group in March 1985 to join the NIH as a tenure-track investigator but continued to contribute heavily to CHARMM. He developed a very fast version for the Star-100 array processor, achieving performance comparable to the Cray-1. In 1986, he developed the PERT facility, which became widely used for free energy calculations. In the late 1980s, he extended Martin Field's CRYSTAL facility to include additional symmetry types and both external and internal virials. In the early 1990s, he added efficient parallel capabilities for small multi-processor systems (around 16 processors). His group analyzed and improved cutoff methods and added PME to CHARMM for more accurate electrostatics. He collaborated with Richard Pastor to develop the Langevin piston algorithm for constant-pressure simulations, which has become widely used. Over the subsequent three decades at NIH, Bernie continued to add new methods and features, leaving a profound legacy on CHARMM's evolution.

### *Force fields*

The first MD simulation of a protein relied on a mixed bag of parameters, many coming from the work of Lifson (18). A few adjustments and improvements, in particular to account for solvation by water molecules, led to the Param19 force field, which was largely an ad hoc collection of parameters from a variety of sources, such as electrostatic parameters based on Wally Reiher's quantum chemical thesis and backbone parameters from thesis of Bruce Gelin combined with parameters from unknown sources. When Jeremy Smith arrived as a postdoc in 1985, Martin initially put him in charge of developing a new force field, and this led to a "pre-new force field" conference abstract (139), followed by a paper by Jeremy and Martin on the geometries and conformational energetics of some classes of organic molecules (140). Martin subsequently assigned amino acid residues to group members. Roland H. Stote entered the Karplus group at Harvard in 1988 with a postdoctoral fellowship for the National Institutes of Health. There he started working on the CHARMM program, implementing methods for treating long-range electrostatic interactions (141) and participated in the group-wide effort to develop the CHARMM all-atom force field with a focus on metal ions (142). Indeed, these two lines of work were not unrelated as Martin recognized that metal ions can introduce important long-range electrostatic interactions. Martin's insight in electronic structure also oriented the development of the force field model toward one that is referred to today as a nonbonded model because of the absence of any specific bonding terms. This was unlike other models being used at the time. The model was first used in the study of the metalloproteins carboxypeptidase A and carbonic anhydrase (142). Alex MacKerell's arrival in the group in 1988 accelerated the force field effort. He became involved in optimization of the nonbonded parameters for small model compounds representative of proteins and other biomolecules, work that was facilitated by Jiali Gao and the Monte Carlo BOSS program from the Jorgensen group. One question immediately to be resolved was the choice of a water model—whether an existing method would be used or an entirely different water model created, such as using the Buckingham potential or different powers for the repulsive term in the Lennard-Jones potential. Extensive tests were conducted by Jiali and Alex, scanning the parameter space and functional forms. In the end, it was concluded that no noticeable benefits could be gained by switching to different functional forms since similar results could be obtained simply by adjusting the values of empirical parameters and partial charges. Keeping the spirit of balancing computational efficiency and accuracy, the TIP3P model was preferred over any new model that could have been derived from this exercise or over the four-point, off-center charge TIP4P water, and it was adopted for the new CHARMM force field. While this represented an important contribution to the force field, this work also led to the observation of the importance of dispersion to the condensed-phase properties of polar liquids (143). Alex's efforts also involved the amino acid histidine and expanded into the nucleic acids and the lipids. Alex gradually assumed overall responsibility and shouldered much of the work for the development of the protein force field effort, with various group members contributing to the optimization of different amino acids and the protein backbone, with a collection of over 25 group members contributing to the effort. While much of the optimization effort was completed by 1990, final adjustments continued until 1992 (144). These efforts ultimately led to the widely used all-atom CHARMM22 protein force field, which had over 16,000 citations as of 2025. While this and the other force field optimization efforts were completed by 1992, in the classic, careful publication approach of Martin, none of the manuscripts were published until well after Alex and the other authors left the group, with the nucleic acid manuscript being published in 1995 (145), the lipid chapter published in the 1996 (146), and the protein paper being published in 1998 (144). This collection of force field parameters, which were optimized to be consistent with each other, allowing for simulations of heterogeneous system, were referred to as CHARMM22. Subsequent work in a variety of labs of former Karplus group members and their offspring led to new versions of the CHARMM additive force field including the current CHARMM36 force field with full coverage of biological molecules as well as small, drug-like molecules. In addition, CHARMM36 was the foundation for the implementation of the polarizable Drude force field (147), which now covers the full range of biological molecules and is being extended to small molecules.

Harmonic models have long been used try to identify conformational changes in proteins, which are of functional importance. Early in the development of bioMD simulations,

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comparisons were made between the full dynamics of polypeptides and the harmonic approximation to their dynamics (32). While it was clear that anharmonicity plays an essential role in the motions of biomolecules, "quasi-harmonic" models, which capture many features of the full dynamics but make use of the machinery of normal mode analysis, occupy an important place in biomolecular modeling. In 1984, a quasi-harmonic approximation was proposed for studying very-low-frequency modes in proteins (33). The force constants of the empirical potential are quadratic approximations to the potentials of mean force; they are evaluated from an MD simulation of a protein based on a detailed anharmonic potential. The method was applied to identify the very-low-frequency modes of BPTI. Conformations corresponding to functional displacements along selected low-frequency modes were analyzed. In subsequent years, "quasi-harmonic" protein models were further developed for use in several different contexts, including to the evaluation of configurational entropies (34,148) and free energies (149) and the calculation of vibrational spectra (150), to name a few.

### *Free energy calculations*

Alchemical free energy methodologies used in biomolecular simulations stemmed directly from the fundamental statistical mechanical developments of Kirkwood on TI (151) and Zwanzig on FEP (152). The concepts were first introduced to realistic molecular systems by McCammon (153,154) and Jorgensen (155), followed soon by applications to biomolecular systems by the groups of Kollman (156,157) and Karplus (55). By the late 1980s, there was much excitement about these new types of calculations, but, soon enough, there were also controversies about a wide range of issues (158). Having joined the Karplus group in early 1991, Stefan Boresch's first task was to investigate the results of a free energy component analysis, the correctness of which had been questioned by van Gunsteren and coworkers (159), by applying it to relative solvation free energy calculations instead of protein-ligand systems. The analysis and reinterpretation of the meaning of a component analysis were completed rather quickly (160,161), but this did not answer the question of how changes in bonded force field terms contribute to free energy differences with different approaches to carry out the alchemical transformation, specifically when using a single versus a dual topology approach (162). The breakthrough came only in 1994, when Boresch "rediscovered" a long-forgotten paper by Herschbach et al. (163), providing expressions for the partition functions of molecular polyatomic molecules in terms of contributions by individual atoms. Reproducing analytical results for model systems by simulations (164) reconciled apparently contradictory results in the literature and paved the way toward the full understanding of how bonded energy terms have to be handled in single and dual topology free energy simulations, respectively.

Martin was not the typical mentor, looking over one's shoulder and being ready to give advice. Instead, he managed to create an inspiring atmosphere that made it possible to exceed one's own capabilities, like a mountain guide who on a difficult climb makes one go the needed extra mile. Being in a large group of excellent young scientists with widely varying backgrounds and expertise was an invaluable resource, as well as quickly learning that one should go into a meeting with Martin only extremely well prepared. However, provided one could back up one's findings, he never minded if one's results contradicted his expectations. Right before his PhD defense (Nov 28, 1996, Strasbourg), Boresch had an hour-long meeting with Martin to go through the bonded terms results in detail, which ended with Martin stating: "Now I see." Understanding the contribution from changes in bonded energy terms turned out to be of direct relevance to the correct treatment of dummy atoms (165) and also led to suggesting versatile restraints for the use in absolute binding free energy simulations (166,167), which are now routinely used (168).

To obtain deeper thermodynamic insight beyond the total free energy change ($\Delta G$), Martin and co-workers introduced methods that decompose $\Delta G$ into its enthalpic ($\Delta H$) and entropic ($-T\Delta S$) components. This decomposition, made possible through approaches such as the finite-difference method and the simplified confinement method (169,170), provides a more nuanced picture of the forces that govern molecular processes. Martin demonstrated the power of this analysis in studies of protein folding, where it reveals the delicate enthalpy-entropy balance that determines folding pathways and stability (171), and in molecular recognition, where dissecting the binding free energy exposes the compensating interplay between favorable interactions (enthalpy) and the entropic cost of restricting conformational freedom, thereby clarifying the driving forces of molecular association (172).

The work on free energy methodology started in Martin's group in the late 1980s (55), followed by the correct treatment of bonded terms and dummy atoms, laid the foundation for the development of rigorous free energy methods, particularly alchemical approaches such as FEP and TI, that now underpin computational biochemistry and have profound implications for computer-aided drug design. These techniques enable highly accurate predictions of relative and absolute binding affinities, solvation free energies, conformational equilibria, and ligand-optimization pathways, thereby creating a direct link between molecular simulations and experimental structure-activity relationships.

### *Reaction pathways and enhanced sampling*

The study of chemical reactions was an early interest in Martin's own career. Accordingly, Martin's Nobel Lecture title was "Development of multiscale models for complex chemical systems: from $H + H_2$ to biomolecules" (3).

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Chemical reaction dynamics of small and large systems also became a central interest in Markus Meuwly's work (173,174). One particular episode goes back to the 2012 CHARMM developers' meeting. After talking about the infrared-radiation-induced photodissociation of sulfuric acid ($H_2SO_4$) (175) and showing a movie of the breakup into $H_2O + SO_3$, Martin said: "I find it remarkable that people are still looking into these trajectories just with much more modern tools. For the first trajectories (from 1965), I had flip-books produced for us to watch the atomic motions." Eventually, Martin had the flip-books made into movies, which he showed during his 2013 Nobel Lecture. Another recollection concerned the role of "simple systems." On one occasion in Martin's Harvard office, listening to an account of high-energy atom+diatom reaction dynamics by Markus Meuwly (176), he remarked: "One cannot overemphasize the value and relevance of *simple systems*: even for the $H + H_2$ exchange reaction, new discoveries are still being made—and will continue to be made." This, of course, was true (177,178). He finished by saying: "Nowadays, people strive to simulate ever larger systems but often forget that superficially simple systems contain beauty and surprises to be discovered."

The identification of reaction pathways for conformational transitions in small molecules was often proceeded by finding the transition states and then following steepest descent paths toward the reactant and product states. Identifying reaction pathways for conformational transitions in proteins proved to be more challenging, even for well-defined local transitions such as the tyrosine ring flip (36). A significant advance was made by Ron Elber in creating a discretized path or Gaussian chain composed of intermediate conformations of the system connecting reactant and product states (179). Bias potentials were used to make the path self-avoiding and to keep the average distance between intermediate states constant. Following an initial guess, the reaction path was optimized by minimizing the underlying potential energy. Elber and co-workers further refined the method for the calculation of steepest descent pathways (180), leading to a remarkable study of transition pathways in a tetrapeptide (181). That later work included the first application of transition disconnectivity graphs to complex molecular systems (182,183). The popular nudged elastic band method (184) and the string method (185,186) evolved from the work by Elber and Karplus. An alternative approach developed by Stefan Fisher during his Ph.D. thesis was "conjugate peak refinement" (187).

Arjan van der Vaart received his MS with Herman Berendsen and his PhD with Kennie Merz; while both groups extensively used MD simulations, Arjan's research had focused on quantum dynamics and semiempirical methods. To finally learn classical simulations, Arjan joined the Karplus group as a postdoc in 2001, first in Strasbourg and then at Harvard. After briefly working on catalytic antibodies, Arjan focused on the GroEL chaperone. Intriguing hydrogen-tritium exchange experiments by Shtilerman, Lorimer, and Englander suggested that GroEL aids protein folding by first partially unfolding misfolded proteins (188). Arjan's simulations of the opening transition of GroEL in the presence of a denatured protein showed that GroEL indeed exerts a force that leads to partial unfolding (189). The simulations also identified the mechanism of unfolding: it was the relative motion of several domains that pulled apart the substrate.

To simulate the opening transition of GroEL, Arjan had used targeted MD (TMD), which reduces the root mean-square deviation with a desired target at every simulation step (190). He realized that TMD is computationally inefficient and artificially cuts through free energy barriers. He solved these deficiencies in an improved version that follows low free energy pathways (191). He then combined this new method with grid searches and one-dimensional adaptive umbrella sampling to efficiently calculate minimum free energy pathways and associated free energy profiles for conformational changes (192). Together with Paul Maragakis, Arjan developed Gaussian-mixture umbrella sampling, which uses Gaussian-mixture models to efficiently escape basins on high-dimensional free energy surfaces (193). The method was used to calculate free energies of Cy3-DNA stacking by enhancing the sampling of five dihedral angles (194) and is a precursor of machine learning approaches in enhanced sampling (195). Arjan's interests in sampling and conformational dynamics were cemented in the Karplus lab (196).

The locally enhanced sampling (LES) approach was developed by Ron Elber to study ligand diffusion pathways in proteins (197). This method illustrates one strength of Martin Karplus, which was his insistence on the connection between theory, simulations, and experiment. His preference for theory was straightforward calculations. On the other hand, he was open to approximate theory if it provided useful insight into experiments. An early challenge in the protein dynamics field was determining the escape pathway of a diatomic from myoglobin. According to the crystal structure (198), there is no pathway from the binding site to the aqueous solution that avoids clashes between the ligand and protein groups. This observation motivated a search for protein fluctuations that open gates. Case and Karplus examined the dynamics of the histidine gate, a spatially short pathway out of the protein matrix (40). However, a question remained whether this is the correct pathway and if a dominant pathway exists. To explore alternative pathways, multiple simulations of the protein and the ligand may be conducted, sampling ligand exits. Such straightforward simulations would undoubtedly be Karplus's first choice. This, however, is an expensive calculation and was not feasible in 1990. Instead, the approximate simulation method of LES was introduced by Elber and Karplus using the following considerations. First, the protein is large, and the ligand is small; therefore, the forces induced by the ligand on protein motions are not

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significant. Second, the gate opens by thermal fluctuations uncorrelated with the diffusion of the ligand. To sample an escape event, the ligand must be near the transiently open gate at the right time. Elber and Karplus introduced a mean-field approximation where the ligand is represented by a discrete number, $N$, of ligand copies, each with a weight of $1/N$. These copies are distributed within the protein, exploring alternative pathways. The copies experience the full force of the protein and exert an averaged force over all copies on the protein. Due to this averaging, the dynamics is approximate. In fact, Straub and Karplus later showed that LES violates equipartition (199). Nonetheless, LES proved to be useful. The statistics of sixty copies was enough to qualitatively identify multiple escape pathways and a network of cavities in thermally fluctuating protein. Observations of several states were later examined experimentally (200) and computationally (201). Karplus acknowledged that, despite the dynamical flaws of LES, its efficient exploration hints at a new concept of multiple pathways and opens new research directions. Many other groups have also utilized LES or improved its properties in follow-up studies (a sample is provided in the references), ranging from simulations of ligand escapes (202–207), structure prediction (208), and free energy calculations, to technological adjustments (203,209–212). Moreover, the paradigm of averaging simulation observables to enhance sampling has been used in the Karplus group for other developments. One example is the momentum-enhanced hybrid Monte Carlo method of Andricioaei, Dinner, and Karplus (213), where continuously updated average momenta are used to bias the motions of the system. This idea stemmed from previous work by Wu and Wang (214), who had proposed a self-guided MD method based on force averaging, and was then developed further by Wu and Brooks in the realm of stochastic dynamics (215).

In January of 1991, Jeff Evanseck joined the Karplus group. Evanseck's PhD dissertation involved quantum studies of pericyclic reactions and Monte Carlo simulations of the organic phenomena. The initial research intent with Martin was to employ a QM/MM approach to identify and assess the chemical factors of enzymatic catalysis, such as substrate polarization, contributing to rate enhancement of triose phosphate isomerase (133,134). However, with the cascade of computer architecture advances in the early 1990s, where picosecond simulations turned into hundreds of nanoseconds seemingly overnight, it became clear that Martin was excited about the future opportunities in solidifying experimental connections, codifying sampling techniques, and generating deeper chemical insight into diverse and heterogeneous ensembles of biomolecules. As a result, their research efforts took a dramatic turn. Along with Dr. Leo Caves, we learned how to extract information from big data by using multivariate analysis to analyze the extent of ensemble generation by multiple short-time and single long-time simulations and started to conceptualize the hierarchy of structure and dynamics of conformational substates (216), as introduced by Frauenfelder and co-workers on protein states and proteinquakes. We ultimately learned that principal component analysis was a useful tool allowing for dimensional reduction and visualization of big data generated by MD. Due to Martin's clarity and vision for research, we continue to pursue research agendas that dissect diverse and heterogeneous RNA ensembles leveraged with experimental data. One life lesson learned was that Martin truly appreciated diversity, where he successfully attracted, welcomed, and nurtured extraordinarily gifted researchers from an all-encompassing range of scientific backgrounds worldwide. His research team of students created a learning environment that was second to none.

A variety of enhanced sampling methodologies were developed in collaborations with Tamar Schlick. In the early 1990s, Tamar was just establishing her mathematical biology research group at NYU and heard about the work of Karplus and his colleagues on MD of biomolecules. To discuss the algorithms she was working on, she presented a poster at the meeting he was speaking at, the 1991 International Academy of Quantum Molecular Science, in Menton, France. Coincidentally, Tamar found herself at the same lovely Villa Genesis hotel, where his wife, Marci, and Martin were also residing, and this presented more opportunities for discussions. Martin encouraged Tamar to try the methods on real biomolecular systems and invited her to attend the CHARMMers meeting in Harvard. This led to work with Bernie Brooks on the truncated-Newton method for multivariate minimization (217) for CHARMM (218). Around the same time, former Karplus trainee Philippe Derreumaux joined her lab as a postdoctoral fellow. Philippe had worked with Martin on protein simulations and was eager to try on proteins the methods Tamar was developing to increase the timestep for MD simulations. The resulting protein studies (219) revealed realistic issues that did not arise in coupled harmonic oscillator systems and led to development of methods for overcoming instabilities and resonances (220,221). This fundamental timestep limit is due to the numerical differentiation algorithms used to integrate the equations of motion. Typical methods used (leapfrog Verlet) become unstable when the timestep is too large relative to the period (around 10 fs) of the high-frequency molecular motions such as light-atom bond vibrations (222,223). An initial attempt using implicit Euler methods was unsuccessful due to damping of other modes, but later methods overcame these instabilities with more complex approaches that also avoid numerical resonance (224,225) and work well in conjunction with fast particle methods (226). Later, as Tamar remained in close contact with Martin throughout her career, they discussed enhanced sampling methods. Because MD can usually sample local fluctuations rather than large-scale transitions and biological reactions, there has been great effort to develop such methods, like LES discussed earlier. Martin was always very appreciative and encouraging of

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the power of rigorous mathematical approaches but emphasized the need to apply them to challenging real-life biological problems. His attention to detail and keen ability to zone into key ideas and issues were remarkable. Although his work focused on proteins, he was also very interested in DNA-associated processes (227). At his urging, Tamar went on to develop protocols that enabled the first application of transition path sampling to biomolecular systems (228). TPS, developed by David Chandler and co-workers Dellago, Bolhuis, and Geissler, is an enhanced sampling method grounded in statistical physics that employs an MD protocol in Monte Carlo trajectory space to generate reactive trajectories in a thermodynamically sound manner (229). However, the protocols for generating reactive trajectories, piecing them together to cover the transition in question, and evaluating the free energy pathway had only been applied to very simple model systems at that time.

With her postdoc Ravi Radhakrishnan, Tamar developed protocols that could be applied successfully to biomolecular systems by piecing together appropriately the successful reactive components of the pathway and developing a new free energy method, BOLAS (230). BOLAS uses the same order parameters as developed for the TPS protocol to generate reactive trajectories that sample the target biomolecular transition to compute free energies for the reaction in an efficient and accurate manner, much better than umbrella sampling methods. The resulting combination of piecing together reactive sub-paths, each generated with different order parameters appropriate for the system, and the efficient BOLAS method to compute reaction free energies, was successfully applied to a DNA polymerase $\beta$ system involved in base excision repair (228). The simulations showed that a cascade of local conformational flips involving amino acid residues of the DNA polymerase protein interacting with DNA complex guide the system from its open to closed states (231). Further, the rate-limiting conformational substep could be identified as an arginine residue Arg258, termed the "gate-keeper." Experiments later confirmed the gate-keeping role and associated high barrier of Arg258 in pol $\beta$ (232), as well as predictions for other polymerases using the same approaches (e.g., noted importance of Arg517 for pol $\lambda$ (233) and preferred G:G *syn* orientation for pol X (234)). The free energy barriers were found to be much higher for an incorrect incoming nucleotide (e.g., A instead of C opposite G) (235). Even more exciting, the same novel sampling and free energy protocols could be applied for bond breaking events (excision repair, where an incoming nucleotide binds to the DNA base) using QM/MM extensions. These studies offered atomic-level details of substeps involved in both the conformational and chemical steps of base excision repair. A recent application of the same protocols in combination with Markov State Modeling revealed atomic-level details of an RNA pseudoknot transition associated with viral frameshifting mechanisms (236).

Martin cited these works in his protein dynamics review with John Kuriyan (237) and often mentioned them to others who were interested in enhanced sampling and who subsequently approached Tamar. In that review, Martin and John commented on the state of MD simulations of proteins in a characteristic understated way: "The combination of increased computer power and improved potential functions has resulted in an ability to generate simulations that approach the point at which they can survive critical examination by the experimentalists who determine the structures of the proteins being simulated" (237). This statement likely also reflected Martin's early experiences from disbelieving experimentalists who often viewed with skepticism computational biophysics work in paper reviews and conferences. No doubt Martin would be delighted today to see experimentalists themselves encountering referee comments requiring them to add computational modeling into their works to probe their conclusions further. Martin would also be very proud of the eclectic generation of computational biologists he has spawned and their impact on biomolecular science today.

### *Multiscale QM/MM simulations*

In the early 1970s, Martin established a collaboration with assistant professor Bryan Kohler prompted by Hudson and Kohler's pioneering discovery of a lowest-lying forbidden state in a substituted octatetraene polyene (238). Following the initial work by Honig (10) and Warshel (11) at the time, no molecular orbital theory in popular use predicted the existence of this forbidden state, and Martin asked his senior graduate student, Klaus Schulten, to explore adding high levels of correlation to the simple Pariser-Parr-Pople (PPP) method. Klaus generated a version of the PPP method with full single and double configuration interaction, and theory indeed predicted the existence of the low-lying forbidden state observed experimentally by Hudson and Kohler (12). Subsequently, Bob Birge joined the Karplus group as a postdoctoral fellow to explore the relevance of correlation in biological molecules such as retinal and the protein-bound chromophores in rhodopsin and bacteriorhodopsin. Using Schulten's PPP method, it was discovered that low-lying forbidden states do exist in the retinals, but the level ordering is conformationally dependent (13). Five years later, Birge and his research group at U.C. Riverside demonstrated that the interaction of the forbidden state with the strongly allowed ionic state was responsible for creating a barrierless photoisomerization surface in the protein-bound chromophore of rhodopsin (239). The early studies of Martin and Bryan's research groups have had implications for biophysical photochemistry far beyond these early studies. The multiscale approach has proved to be a powerful principle, a pragmatic approach to modeling complex processes in large systems, which would otherwise be beyond the reach of detailed calculations. One driving

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motivation was to allow quantum chemical calculations on (small regions of) proteins, while including the effects of the whole system. Practical applications required methodological development and testing. The implementation of the semiempirical QM method AM1 within the CHARMM program (240) was the first practically useful, and widely applied, QM/MM approach for biological systems, and it served as a model for future developments. Martin demonstrated it in investigating the mechanism of the enzyme TIM (see above).

Hong Guo first met Martin during Martin's visit to Beijing, China in 1982, after receiving his MS degree from Jilin University based on research on density matrix theory with Professor Aoqing Tang. He joined Karplus group in 1983 as a graduate student in the Chemical Physics program. Initially, Martin asked him to learn the extended PPP-CI method from Russell Hemley and Bernie Brooks and to study polyenes. Although conjugated molecular systems were still a part of Hong's PhD research, the method used was *ab initio* quantum mechanics (Gaussian). Guo also used *ab initio* QM methods to study hydrogen bonding properties and effects of hydrogen bonds on structures and energetics of N-methylacetamide, a model for the protein peptide bond, and related complexes. The results from the *ab initio* calculations demonstrated that the hydrogen bonding interactions involving N-methylacetamide could be highly cooperative and have some important structural and energetic consequences (241,242). It was suggested that it might sometimes be necessary to introduce corrections to the pairwise additive potentials used in most simulations and that a force field that changes the internal parameters as a function of external perturbations might be required; charge polarization was one, but not the only, effect of this type. (241,242).

Guo joined the Karplus group for a second time, as a postdoctoral scientist, in 1998, after spending several years in Canada. Martin suggested that he should learn the QM (SCC-DFTB)/MM method from Qiang Cui and collaborate with Professor Bill Lipscomb and Qiang Cui to study chorismite mutase. The study led to two publications, one for yeast chorismate mutase (243) and one for Bacillus subtilis chorismate mutase (244). The results provided strong evidence for the hypothesis that chorismate mutase uses conformational optimization of the substrate to lower the transition-state barrier. It indicates that, in contrast to the induced fit model, enzymes may induce conformational changes of substrates, with substrate structures changed along the reaction coordinates toward the transition state for enzyme-catalyzed reactions.

Annick Dejaegere entered the Karplus group at Harvard in 1989, with a postdoctoral fellowship from the Belgian National Fund for Scientific Research. Her interest was in learning QM-MM methodologies and understanding enzyme reactivity. Martin suggested that she focus on the reactivity of phosphates with the long-term goal of implementing QM-MM methods for studying enzyme-catalyzed phosphate hydrolysis. She started working on the reactivity of small phosphates, together with Carmay Lim and then also a postdoc in Martin's group. Annick's work questioned a long-standing hypothesis on phosphate reaction pathways (245) and pointed to the essential role of solvation in the reactivity of phosphates (246). From 1992, Annick worked in the Karplus group in Strasbourg, where she pursued reactivity studies with Xavier Lopez (University of the Basque Country), followed by a postdoc in Strasbourg, and worked with Nathalie Reuter on the analysis of frontier bonds in QM/MM simulations.

Darrin York joined the Karplus group at Harvard in 1996 as an NIH postdoctoral fellow and moved to the Strasbourg laboratory in 1997 as an EMBO postdoctoral fellow. At that time, the development and application of multiscale quantum models were areas of intense activity, with particular emphasis on modeling chemical reactions in complex condensed-phase environments. Rigorous treatments of long-range electrostatic interactions in QM/MM simulations had not yet been realized (247,248), and solvent effects were often represented using continuum models.

One such approach employed boundary element methods integrated into QM calculations through a reaction-field formalism. However, in conventional boundary element solvation models, forces (energy derivatives) were typically treated approximately and exhibited singularities arising from discontinuities in the electrostatic potential. To address these limitations, York and Karplus developed a smooth boundary element solvation potential (249) from which analytic derivatives could be obtained, enabling stable geometry optimizations, transition-state searches, and event-driven MD simulations. These methods were initially applied to studies of phosphate diester reactivity in collaboration with Xavier Lopez, Annick Dejaegere, and Fabrice Leclerc (250,251), and they were subsequently adopted more broadly in a range of quantum chemistry software packages.

When Reuter joined the Karplus group in Strasbourg, she had just started her PhD in Nancy—a mere 100 miles west of Strasbourg, in the Theoretical Chemistry Laboratory led by Prof. Jean-Louis Rivail. She was working with the local self-consistent field (LSCF) formalism (252), in which QM/MM frontier bonds were described using strictly localized orbitals, inspired by the work of Warshel and Levitt (253). The LSCF formalism, developed in the group in Nancy for QM calculations at the MNDO semiempirical level (252,254), was an attractive alternative to the so-called link atoms as it avoided introducing an extraneous atom along covalent bonds separating the QM from the MM regions. Link atoms were introduced along frontier bonds to satisfy the unsaturated valences, and that formalism had been implemented in CHARMM by Martin Field (240). The LSCF formalism was implemented in a code (GEOMOP) limited to the calculation of energy and forces on QM atoms under the influence of fixed MM charges.

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Nathalie linked GEOMOP and CHARMM and compared results obtained with the LSCF formalism to various ways of defining the link atoms and to reference QM calculations. The work was done with Annick Dejagere, and they showed that frozen orbitals and link atoms could be used reliably provided a number of precautions were taken in the choice of the QM region and of the frontier bonds, as well as in the treatment of interactions between QM and MM atoms in the frontier region. (255).

Aaron Dinner was a Burroughs Wellcome Fund Hitchings-Elion Fellow nominally working with W. Graham Richards at Oxford University when Martin held the Eastman Visiting Professorship there in 2000. Aaron was interested in broadening his experience in molecular simulation beyond protein folding, and Martin suggested looking at solvent effects in chemical reactions; in turn, he introduced Aaron to G. Michael Blackburn, who, with John Tainer, had recently obtained a crystal structure of the DNA repair enzyme uracil DNA glycolsylase bound to DNA (256). In the structure, the C1 position, where the base is attached to the sugar, exhibited a tetrahedral geometry, rather than the usual trigonal planar one, and it was suggested that strain facilitated catalysis. Martin was skeptical of this mechanism, and calculations quickly suggested that the nucleotide was in an unstrained tautomer (257). However, simulating the reaction remained challenging because it was not clear how to treat the solvent shielding of the DNA backbone because averaging over solvent configurations was prohibitively computationally costly at the time. Ultimately, building on a charge-scaling/continuum-electrostatics procedure that Tom Simonson and Georgios Archontis developed with Martin for treating electrostatics in free energy simulations (258,259), Aaron and Martin were able to simulate the reaction and discovered that, surprisingly, desolvation of the phosphate groups in the backbone upon DNA binding accounted for most of the catalytic effect (257). This mechanism was subsequently experimentally validated by experiments in which methylphosphonates were substituted at specific positions (86,260). Aaron and Martin both went on to study DNA repair further (82,84,85,87,261–263).

Qiang Cui joined the Karplus group as a postdoctoral fellow in 1997, after receiving a PhD with Keiji Morokuma on quantum chemistry methods and application to small molecules. For a research topic, Martin suggested the analysis of proton tunneling in enzymes using variational transition state theory. With little experience in biochemical systems, Qiang developed a number of cluster models to explore the relevant potential energy surfaces using density functional theory, which consumed the computational allocation of the Karplus group at Argonne in two weeks. Martin was surprised at this consumption and asked Qiang to write a detailed report on what he was working on. After reading the report, Martin was more relaxed and supported Qiang to apply for more computational resources, at NERSC. Qiang had the opportunity to attend several small-scale workshops that featured leading enzymologists such as Judith P. Klinman, from whom he learned the latest kinetic isotope measurements on various enzymes. During his PhD studies, Qiang read quite a few papers about the reaction path Hamiltonian approach (264) and its numerous extensions. This motivated him to analyze how various vibrational motions are coupled to proton transfer reactions in enzymes and to research the temperature dependence of kinetic isotope effects (265,266). While studying QM/MM methods, Qiang realized that most studies were limited to the calculation of potential energy for reaction path and MD simulations. Trained in the area of quantum chemistry, Qiang thought that QM/MM methods should also be applied to compute various experimental observables, such as vibrational spectra (267) and NMR chemical shifts (268). Martin was very supportive of this and encouraged him to pursue these directions, as the effort paralleled his earlier work at the pure QM level on the effect of "weak" intermolecular interactions on molecular properties (269). Qiang also explored the analysis of intermolecular interactions and their impact on chemical reactions by embedding high-level QM descriptions into low-level QM methods (270).

The multiscale approach has proved to be a powerful principle, a pragmatic approach to modeling complex processes in large systems, which would otherwise be beyond the reach of detailed calculations. One driving motivation was to allow quantum chemical calculations on (small regions of) proteins, while including the effects of the whole system. Practical applications required methodological development and testing. The implementation of the semiempirical QM method AM1 within the CHARMM program (19–21) was the first practically useful, and widely applied, QM/MM approach for biological systems, and it served as a model for future developments. Martin demonstrated it in investigating the mechanism of the enzyme TIM (134). Inspired by Martin's work on TIM, and the potential of the QM/MM method, Adrian Mulholland visited the Karplus group in Harvard in 1993 during his PhD studies with Graham Richards. Having learned of the QM/MM approach, and having applied it to enzymes and DNA and having experienced the stimulating environment of the Karplus group, Mulholland returned as a postdoctoral Wellcome Fellow. With Martin, he applied correlated *ab initio* methods (which can provide high accuracy and had by then been interfaced with CHARMM) in QM/MM calculations, identifying the enolate of acetyl-CoA as the nucleophile in citrate synthase, and he showed how it is stabilized by hydrogen bonding at the active site (271).

## Drug design

The Karplus group pioneered the use of molecular fragments to map protein binding sites. Indeed, a 1991 paper by Andrew Miranker and Martin on the simultaneous minimization of multiple copies of small and mainly rigid

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functional groups in the energy potential of the protein can be considered as the first fragment-based procedure for drug discovery (272,273). The method, partly inspired by the LES approach developed by Ron Elber (197), was named "multiple copies simultaneous search" (MCSS), because in the method during minimization (or MD) the interaction energy between multiple replicas of the small molecules is switched off so that each replica feels only the force field of the protein. The surface of a protein is flooded with small molecular probes to computationally identify fragment-binding positions or hotspots. It is interesting to note that MCSS, a computational method for generating functionality maps, preceded experimental methods for generating functionality maps, e.g., by protein X-ray crystallography or NMR spectroscopy, which were introduced in the mid-1990s (274,275). When Amedeo Caflisch joined the Karplus group in September 1991 as a postdoc, he applied the MCSS procedure for the identification of inhibitors of the aspartic proteinase of the AIDS virus (273), which was a very active field of research. This was the first paper by Martin Karplus published in the *Journal of Medicinal Chemistry*, a premiere of which he was quite proud. In the past 35 years, many successful applications of MCSS to proteins (276–278) and RNA targets (279) have been published. MCSS and similar approaches (280,281) had a significant impact on the field of fragment-based drug design (282–285).

Inspired by the MCSS procedure, the Caflisch group at the University of Zurich developed in 1999 a program for high-throughput docking of rigid fragments called SEED (Solvation Energy for Exhaustive Docking) (286). In contrast to the energy minimization used in MCSS, SEED performs an exhaustive search in a discrete space defined by rotations around individual protein/fragment hydrogen bonds and/or hydrophobic contacts. Another difference is that the electrostatic solvation energy in SEED is evaluated by the generalized Born equation with numerical calculation of the Born radii (287). As in MCSS, the SEED binding energy is based on the CHARMM force field, which is transferable and does not require any fitting parameters. Thus, in contrast to machine learning tools, which are data hungry (288), MCSS and SEED can be employed for protein targets for which inhibitors have not been identified. Successful fragment screening campaigns by SEED have been reported for several targets (289,290).

The simulation protocol called SILCS (Site-Identification by Ligand Competitive Saturation) developed in Alex MacKerell's group in 2009 builds on the MCSS approach, making use of MD simulations of multiple copies of different small molecules in explicit aqueous solution that compete with each other, with water, and for the protein or RNA target (291). A few years later the MD-based sampling protocol in SILCS was extended by an iterative procedure that includes cycles of MD and grand-canonical Monte Carlo sampling (292). In SILCS the small molecules interact with each other as well as the protein, though long-range attractive interactions between specific pairs of solute types (e.g., hydrophobic or ion pairs) are eliminated by a repulsive interaction between pairs of solutes that are closer than a threshold distance. This simulation stratagem makes possible the use of a high concentrations of the small molecules that would otherwise aggregate in the simulation box and *in vitro*. A major advantage of SILCS is the precomputed ensemble of the distribution of functional groups around a macromolecule, based on the small molecule types, that may be converted to free energies that allows one to rapidly study the interactions between proteins, RNA, and small molecules in aqueous conditions to predict a range of properties of utility in drug design.

A cautionary tale of misled drug design emerged about a new class of drug targets called immunophilins in the late 1980s. Martin's young colleague Stuart Schreiber had arrived from Yale, determined to achieve the total synthesis of a natural product with profound immunosuppressive activity called FK506 and the cloning of its receptor. Both goals were achieved by 1990 (293,294). What appeared a remarkable case of convergent evolution, the receptor for FK506, the FK506 Binding Protein (FKBP) and cyclophilin, the receptor for another immunosuppressive drug, cyclosporin A (CsA) both possessed the same catalytic activity—*cis-trans* isomerization of prolyl peptide bonds. It was thought then that inhibition of this common catalytic activity by the two drugs must somehow cause immunosuppression.

Meanwhile, Martin and Stuart were engaged in a new commercial venture, led by former Merck chemist Dale Boger, called Vertex Pharmaceuticals. Their aim was to harness molecular and structural biology and computational chemistry to rationally design drugs, their first challenge being to design a molecule better at inhibiting FKBP than FK506. To do this, the structure of FKBP needed to be solved. Vertex was determined to do this, and a race ensued, bringing in structural groups from Abbott labs and Merck, but also Martin and Stuart themselves, a story recounted from Vertex's perspective in the book *The Billion Dollar Molecule: The Quest for the Perfect Drug* (295). Stephen Michnick had arrived in Martin's group as a postdoc in the fall of 1989, a protein NMR spectroscopist keen to learn theoretical chemistry. Martin convinced him to take on the solution structure of FKBP by then novel multi-isotope multi-dimensional NMR spectroscopy with Stuart's student Mike Rosen. Within a year, Michnick and Rosen succeeded and published their results (296) (297), and a crystal structure of the FKBP-FK506 complex solved by Greg Van Duyne, Andy Karplus, and John Clardy, using molecular replacement phasing based on Michnick and Rosen's solution NMR structure (298) in *Science*. Vertex reported their solution structure a mere week later in *Nature* (299). It turned out that none of this really mattered because inhibition of prolyl isomerase activity by the immunophilins being causative of immunosuppression was a red herring, having

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nothing to do with the immunosuppressive activities of FK506 or cyclosporin A.

Postdoc Jun Liu in Stuart's group discovered that the real convergent mechanism of immunosuppression of FK506 and cyclosporin A was their binding, in complex with their receptors, to a common signaling enzyme, the protein phosphatase calcineurin (300). The discovery of novel mechanisms of action of FK506 and CsA inspired the new pharmacology of molecular "glues" and PROTACs as strategies to inhibit "undruggable" targets (301), (302). Michnick learned two things from this experience: first, that chemical assumptions should never be put before biological observation and, second, that discovery of biological mechanisms could begin with discovering novel protein-protein interactions. The latter inspired him to invent Protein-fragment Complementation Assays (PCAs), to study the dynamics of protein-protein interaction networks as a means to measure effects of environmental and genomic variation on cellular processes and discover mechanisms of signal transduction and other processes (303), (304), (305), (306), (307), (308). Coincidentally, today PCA is also being used as a complementary method to computational approaches, to accurately measure free energies of stability, protein-protein interactions, and allosteric effects of thousands of mutant protein variants (309), (310), (311).

In the late 1980s, work in the Karplus group included the use of computational approaches to deepen our understanding of how enzymes function, going beyond what was possible experimentally. Diane Joseph-McCarthy joined the Karplus group in 1986 as a joint PhD student with Gregory Petsko at MIT. She was excited to explore and develop computational approaches to study the dynamics and reactivity of enzymes as a means of elucidating their function. In her first paper in graduate school, Joseph-McCarthy used MD together with structural analyses to characterize the loop movement in the enzyme triosephosphate isomerase, showing that the loop acted as a lid to shield the active site (312).

Joseph-McCarthy then worked with Martin Karplus as a postdoctoral fellow, jointly with James Hogle at Harvard Medical School, on the refinement of MCSS and its application to viral and immune targets. In one application, MCSS was applied to the poliovirus capsid protein for fragment-based design of antiviral compounds (276,313). For the next twenty years, while working in the pharmaceutical industry, Joseph-McCarthy saw computational techniques for fragment positioning, virtual screening, MD, and FEP come to be commonly applied to drug discovery problems. PhDock used fragment-positions derived from MCSS calculations to accurately dock small molecules into protein binding sites to identify early hits (314,315). Fragment-based drug discovery and structure-based lead optimization, utilizing many of the foundational approaches developed in the Karplus group, saw numerous successes (316–318). Upon returning to academics, Joseph-McCarthy has continued to be influenced by the past and current work of Karplusians. Combining physics-based approaches with machine learning is at the forefront. Recent efforts using FTMove, which applies a fast Fourier transform approach to identify hotspots on the surface of ensembles of protein structures, have been applied to SARS-CoV-2 (319). E-FTMap has been developed to enable fragment-based generation of pharmacophores (320,321). Optimal methods for using AI-based AlphaFold (AF) protein models (322) for hotspot mapping (323), cryptic site identification (324–326), and molecular docking have been developed. The generation of realistic protein conformational ensembles is being explored. The development of large language models together with methods combining protein-protein docking for enhanced sampling with AF refinement for predicting antibody-antigen binding is in progress.

Martin's ability to successfully enter new research fields and contribute with pioneering findings inspired also Amedeo Caflisch (postdoc at Harvard during 1992–1994), who at the age of 50 started to establish experimental activities in his research group at the University of Zurich. The goal was to efficiently identify small-molecule ligands of proteins of pharmaceutical interest. The strategy for ligand identification is based on high-throughput docking and *in vitro* validation. More in detail, *in silico* screening by force field-based docking was followed by a full range of wet biochemistry techniques from the gene of the protein target to crystal structures. This multidisciplinary approach has resulted in a large number of potent and selective small-molecule ligands of proteins involved in cancer (327–330) and neurodegenerative diseases (331) and nearly 400 high-resolution crystal structures deposited in the PDB in the past decade (288). It is important to note that the vast majority of these crystal structures of tyrosine kinases, proteases, bromodomains, and proteins involved in epitranscriptomics (m6A-RNA writer enzyme and reader proteins) contain a ligand that was either identified by docking using the CHARMM force field with implicit solvent (286) or a derivative of a fragment identified by docking (289,332).

## Relation to experiments

One of Martin's bedrock principles was that the weight assigned to a theory or model is proportional to its ability to reproduce experimental results. Of course this principle was hardly unique—as Max Planck famously opined, "Experiments are the only means of knowledge at our disposal (333). Everything else is poetry, imagination." Martin's Harvard colleague Frank Westheimer demonstrated that theory is sometimes a lagging indicator, tracking the best experimental results (334). Nonetheless establishing the concordance between simulation and experiment forms an indelible thread through Martin's work. This became particularly important after the initial MD and normal mode calculations were performed in the 1970s, as

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experimental validation of the calculated dynamics was imperative.

### *Nuclear magnetic resonance*

Martin had a special interest in magnetic resonance as a uniquely versatile experimental probe of matter. Possibly kindled during his time at Caltech, this interest certainly blossomed during his time in Illinois, where his colleagues Charles Slichter and Herbert Gutowsky discovered the scalar "J" coupling between nuclear spins (335). Martin's leverage of quantum theory to explain the geometrical correlates of the magnitude of vicinal (3-bond) couplings was a notable triumph of quantum theory, and it is enshrined in the "Karplus equation" (5). His special affinity for magnetic resonance is also visible in the treatment devoted to the subject in the book *Atoms and Molecules* coauthored with Richard Porter (336), and the extensive studies on the prediction of protein NMR spectral features from early MD simulations of BPTI and metmyglobin lasting between 100 and 300 ps (337).

Martin's emphasis on the importance of establishing close connections between theory, computation, and experiment had a large impact on Ron Levy's work with Martin in the late 1970s and early 1980s and his work during this period inspired by Martin. NMR relaxation experiments can provide information about the extent of internal protein motions on this timescale. The effect of very fast internal motions is to increase the spin-lattice relaxation T1, reduce NOEs, and average vicinal coupling constants, by a scale factor determined by angular correlation functions that can be computed from the protein MD simulations (338–343). Using a "model-free" approach to the interpretation of $^{13}C$ NMR data (344), it was shown that the generalized order parameters calculated from the simulations agreed well with corresponding values extracted from experiment, confirming that the relative flexibility of residues were correctly captured by the MD simulations (345). Karplus' interest in connections to NMR experiments in this period was further evidenced by his mentorship of two MD-PhD students at Harvard Medical School, David J. States and Michael A. Weiss. States developed a "pure-phase" method for enhancing the resolution and sensitivity of two-dimensional NOESY spectra (in the course of collaborative studies of BPTI co-advised by the late Christopher M. Dobson) while Weiss developed NMR methods for the sequential assignment of DNA with application to the phage-lambda operator.

When Karplus hosted Richard R. Ernst during his sabbatical at Harvard in 1988, they decided to start a collaboration between their labs by sending one of Ernst's graduate students to Harvard and one of Karplus's graduate students to ETH Zurich. This is how Rafael Brüschweiler got the opportunity to spend the spring and summer of 1989 time in the Karplus group learning about MD and CHARMM, and Benoit Roux spent the following summer at ETH becoming immersed in experimental NMR. At that time, MD was pursued predominantly in the United States with only few MD groups in Europe and none at ETH. An intriguing biomolecule studied in great detail by NMR in the Ernst lab was the cyclic decapeptide antamanide. Due to its compact structure, antamanide behaves like a "mini-protein" with a well-defined overall tumbling behavior while undergoing a wide range of internal motions from proline puckering on the picosecond timescale to phenylalanine side-chain dynamics on the nanosecond timescale and backbone conformational transitions on millisecond timescales. One of the projects Benoit and Rafael pursued was to generate (at the time) a "very long" 800-ps MD trajectory of antamanide in explicit chloroform solvent over the course of several months followed by detailed analysis for comparison with a wealth of NMR data (346). The back-calculation of NOESY and ROESY cross-relaxation rates from the trajectory offered useful insights into the competitive effects of radial versus angular order parameters describing the internal motion of $^{1}H$–$^{1}H$ internuclear vectors. Rafael quickly learned to appreciate the kind of realistic structural dynamic information MD can provide going well beyond the simplistic analytical motional models often used at that time for the interpretation of NMR data. Throughout the years and to the present day, the exploration of the synergies between MD and NMR has remained an integral part of Rafael's research.

The advent of sophisticated isotope labeling strategies and corresponding NMR pulse sequences developed by Lewis Kay enabled the measurement of the dynamics of side-chain methyl groups in proteins. Robert Best had been using these experiments during his PhD with Martin's collaborator Prof. Jane Clarke in 2001, and this caught Martin's interest because he was interested in what types of protein motion the new experiments were sensitive to. Best worked with Martin and his group in Strasbourg to perform MD simulations of the two proteins he was studying, separating the order parameters computed from simulations into contributions from fluctuations within a minimum versus hopping between rotameric states. Both experiments and simulations revealed that protein hydrophobic cores are highly dynamic, and many side chains have significant minor rotamer populations (347)—illustrating Martin's belief that even quite straightforward simulations could yield insights into the aspects of protein dynamics probed by experiment. Martin was also curious about a proposal at that time that an apparent trimodal distribution of published side-chain order parameters reflected different "tiers" in a hierarchical protein energy landscape. By performing simulations for proteins for which experimental side-chain order parameters had been recorded, it was possible to show that, to the extent there was a trimodal distribution, this mainly reflected which side-chain torsion angles were able to isomerize versus being in a single rotamer (348). The

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experience of working with Martin taught Dr. Best not to shy away from tackling controversial issues, as this is often where progress in science is made.

After earning his D. Phil. in Christopher M. Dobson's lab at Oxford, where he studied the conformational states of hen lysozyme by solution NMR spectroscopy, Matthias Buck joined the Karplus lab in 1995. To master "the tools of the trade," he pursued an ambitious project showing that the CHARMM22 force field could reproduce hydrogen-bond geometries in solvated N-methylacetamide (NMA) and lysozyme, and—out of curiosity—in NMA dimers and trimers in vacuum (349). Remarkably, CHARMM22 performed well, even without an explicit hydrogen-bond term; its accuracy arose from the interplay of van der Waals and electrostatic forces. He next used hen lysozyme to study local peptide-plane fluctuations and the dynamics of amide N–H bonds relative to the peptide plane (350). Potentials of mean force and cross correlation analyses closely matched experimental data, underscoring the force field's reliability. This work expanded to simulations of hydrogen-bond breaking in helices and $\beta$ sheets at high temperature, revealing a stepwise unfolding through 3-ten- and turn-like intermediates—a mechanism later confirmed by others. These results, along with related studies on amyloidogenic lysozyme, were never published.

Buck's research on MD trajectories then converged with his earlier experimental NMR studies. A presentation at the 1998 International Conference on Magnetic Resonance in Biological Systems in Tokyo spurred him to complete a comparison between a 1.6-ns lysozyme simulation using the CHARMM22 potential (with CMAP correction) and experimental order parameters measured in Oxford. The work, done with Richard Pastor at NIH and later joined by Alex MacKerell (351), produced one of Buck's most cited papers—and a lasting friendship with both. Buck left the Karplus lab in 1999 for a second postdoc with Michael Rosen, returning to experimental NMR and hydrogen-exchange studies. Since 2002, as a faculty member at Case Western Reserve University, he has united computational and experimental methods in his own laboratory.

### *X-ray diffraction*

MD simulations in the modern era have become an indispensable tool used in the course of refining structures from X-ray and cryo-EM data. In the first application of this kind 40 years ago, the effects of anisotropy and anharmonicity on protein crystallographic refinement of met myoglobin were analyzed (352). Simulations (42,352) were used to generate time-averaged diffraction data at 1.5 Å for comparison with experiment. A systematic deviation between the simulations and experiment was observed, which was attributed to the use of a single-site isotropic model for atomic fluctuations commonly employed at that time. Related studies, temperature-dependent MD of a DNA hexamer, and restrained X-ray refinement simulations, concluded that low-temperature simulations provided a useful tool for optimizing the refinement of X-ray structures (353).

In the early days of protein MD simulations, researchers often compared atomic positional fluctuations from simulations with those inferred from X-ray crystallographic temperature factors. Although these quantities showed reasonable correlations, notable discrepancies were also evident. A central question was whether the treatment of atomic motion in crystallographic refinement—modeled as isotropic, harmonic displacements around mean positions—introduced errors that obscured the comparison. To address this, Kuriyan, Petsko, Levy, and Karplus adopted a theoretical strategy (51). Instead of analyzing actual experimental data, they generated simulated X-ray diffraction data from MD simulations of a protein and refined a structural model against the data using standard crystallographic procedures. The refined structures were then compared with the known average structures and mean-square fluctuations from the simulations. Because the comparison was entirely theoretical, it avoided complications arising from experimental noise, crystal disorder, or measurement inaccuracies.

In their original study, short simulations (25 ps and 300 ps) of myoglobin were used to generate time-averaged diffraction data at 1.5-Å resolution. The PROLSQ refinement program developed by Hendrickson and Konnert (354) was used to refine models against these simulated data. Substantial deviations were observed between the refined atomic positions and fluctuations and those calculated directly from the simulations. The discrepancy arose from the single-site isotropic model used to represent atomic motion in the crystallographic refinement. Many atoms occupy multiple conformational substates, yielding probability distributions with several peaks, yet refinement captures only one, neglecting the rest and producing systematic errors in positions and temperature factors. This insight, first revealed in those early studies, has since become widely appreciated. Recent work (355) demonstrates that accurately modeling conformational heterogeneity in crystal structures reveals how proteins offset the entropic cost of ligand binding, thereby enhancing affinity.

As a new academic at the University of York in the early 1980s Rod Hubbard helped the crystallographer Guy Dodson and the visiting Dorothy Hodgkin with figures for a major paper on the first insulin structure (356). This sparked an interest in molecular graphics, and he wrote new programs for analyzing protein structures on a microcomputer (357). The Dodson group determined structures of many insulins with conformational changes, and he added a feature to flash between structures. This was presented at a lecture at the UK Daresbury Laboratory in 1983 that Martin attended. Rod was struck by Martin's enthusiasm for the images and arranged for a sabbatical at Harvard, arriving in March

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1984. Rod had free access to the Evans and Sutherland graphics in the Gibbs building basement hosted by a VAX 11/780—resources then unimaginable in York. There followed a gloriously productive six months where Rod wrote HYDRA (Harvard York DRAwing program) with heads for graphical displays, including animations of dynamics trajectories. The group was vibrant—Charlie Brooks, Bernie Brooks, Lennart Nilsson, Carol Post to name a few—and they and the interactions with faculty such as Jeremy Knowles and Don Wiley encouraged Rod's creativity. The software was commercialized by Polygen in 1986 (becoming QUANTA) alongside CHARMM. Rod visited Harvard every few months until he acquired equivalent hardware in York. In 1991 a short Harvard sabbatical funded by Martin and Don Wiley resulted in the program HOOK (358) with Mike Eisen, linking functional groups from Andrew Miranker's MCSS (272). These experiences started Rod's interest in fragment and structure-based drug discovery, which he continued at York and in the company Vernalis.

### *Neutron scattering*

Jeremy Smith collaborated as a graduate student with Martin in the early 1980s, calculating neutron scattering quantities from normal mode calculations of BPTI performed by Bruce Tidor and Bernie Brooks. This was an attractive approach to validating simulated dynamics as neutrons are direct probes of amplitudes and frequencies of atomic motions. The results showed that the normal modes would only produce agreement with experiment if the low-frequency vibrations were assigned specific frictional damping properties and if calculated with "switch" style nonbonded truncation (359–361). Later MD work during Jeremy's postdoc at Harvard showed that the temperature-dependent "glass" transition in protein dynamics as observed with neutrons also occurred in MD (362). The general simulation:neutron combination has since become a common approach to investigating soft condensed matter dynamics.

### *Between theory and experiment: The X-PLOR program*

Dr. Axel Brunger first met Dr. Karplus in 1981 when interviewing for a postdoctoral position. Dr. Brunger was keen to learn about MD simulations and simulate proton transfer in bacteriorhodopsin, inspired by Dr. Brunger's PhD advisor, Dr. Klaus Schulten. He was delighted when Dr. Karplus invited him to join his group. However, since a high-resolution structure of bacteriorhodopsin was unavailable at the time, Dr. Karplus suggested a MD simulation of ribonuclease A based on crystal structures that Dr. Gregory A. Petsko had recently determined. Dr. Karplus's suggestion led to a most fruitful collaboration with Dr. Charles L. Brooks III (363). After two years at the end of his postdoctoral fellowship, Dr. Brunger returned to his native Germany, working jointly in the laboratories of Drs. Dieter Oesterhelt and Robert Huber at the Max Planck Institute of Biochemistry. During this time, Dr. Brunger also initiated a fruitful collaboration with Drs. Marius Clore and Angela Gronenborn, combining MD simulations with distance data obtained from solution magnetic resonance experiments (364), which resulted in the creation of the X-PLOR program. It rapidly became obvious that this was going to be a powerful method for refining structures from NMR data (365,366).

Considering the limited opportunities for computational research in Germany at the time, Dr. Brunger decided to pursue an academic career in the United States, and he was grateful when Dr. Karplus offered him the opportunity to return to his group in 1984. During this second period in Dr. Karplus's group, Dr. Brunger explored new applications of "simulated annealing" MD simulations in combination with X-ray diffraction data, in collaboration with Drs. Gregory A. Petsko and John Kuriyan (367), which he also incorporated into the X-PLOR program. This new approach contributed to the growth of the field of structural biology since it enabled more efficient completion of "structure refinement," a method which had previously taken months to complete.

In 1992, Roland Stote, along with Annick Dejaegere, joined Martin on a six-month sabbatical stay at the University of Strasbourg, France, where Martin had been invited by the NMR spectroscopist Prof. Jean-François Lefèvre and chemist Prof. Jean-Marie Lehn (Nobel Prize in Chemistry 1987). After that stay, Martin was encouraged by both Jean-François Lefèvre and Jean-Marie Lehn to set up a laboratory. Toward that goal, both Annick and Roland returned to Strasbourg in 1993 to help in its establishment. There, in close proximity to the experimental teams of Jean-Francois Lefèvre and the crystallography team of Dino Moras (IBMC at the time), collaborations between the Strasbourg Karplus group and experimentalist teams at the University and beyond were established. This set a path for Roland as he became interested in bringing the modeling techniques closer to experiments and interpret experimental data integrating both structure and dynamics. The applications carried out over the years focused on local folding of small disulfide proteins (368), small therapeutically oriented peptides (369), nuclear receptor proteins (370), and enzyme design (371), among others.

## SOME PERSONAL COMMENTS

Working in the Karplus group was a unique experience for many of us, both in the freedom given by Martin in pursuing one owns avenues of interest and in his rigorous scrutiny of these avenues before considering them worthy, which forced us to think harder and deeper. The opportunity to meet, learn from, and remain connected with so

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many dedicated scientists was also a lifelong treasure from having been a Karplusian. Martin continued to support the Karplusians long after they left the group by sharing his critical views on various research topics, and even many years after they had worked in direct contact with him, many of Martin's scientific collaborators kept a strong personal bond with him. This final section collects some personal reflections.

*Axel Brunger:* I attribute my experiences working with Dr. Karplus to profoundly shaping his scientific career. Martin provided encouragement in exploring new ideas and being an excellent mentor, leaving me a lot of freedom to pursue new directions while offering tremendous support and scientific insight. These experiences shaped my subsequent independent career, and I continued to develop methods that combine computer simulation and experimental data. I often moved into new fields and used emerging scientific methodologies to study biological systems, including transitioning my laboratory to performing experiments.

*Lennart Nilsson:* The scientific environment in the Karplus group in the early 1980s was very stimulating, not least by offering a great learning opportunity through constructive interactions with Martin and with the many talented co-workers in the MB-9 area of the Mallinckrodt lab (centered around the coffee machine—very important for a Swede). This formed the basis of lifelong scientific networks that have been reinforced by the annual CHARMM developers' meetings and, most importantly, personal friendships, for which Lennart and Anna are very grateful.

*Markus Meuwly:* I still remember the discussions in which a seemingly unimportant finding got Martin excited, and substantial additional simulations and analyses needed to be carried out. It then often turned out that these aspects provided the essential links between experiment and simulations, which also became a main focus in my subsequent career.

*Adrian Mulholland:* Working in Martin's group was invigorating and led me to a career in research. Martin's vision, of analyzing life at the molecular level, revealing complex dynamics and mechanisms, inspired us all. He had tremendous drive, with groups in both Harvard and Strasbourg, and making time to meet us all and talk through projects and plans in forensic detail. As well as science, he had rich interests in food, cooking, wine, and photography.

*Hong Guo:* I benefited considerably from the training by Martin on how to think scientifically, examine data rigorously, and communicate clearly, as well as much support from Martin during his career.

*Alex MacKerell:* Martin brought together a highly motivated collection of young scientists and directed them to work together in unanticipated ways producing insights and creating computational tools that have widely impacted the fields of computational chemistry and biophysics, and those interactions also created friendships that have lasted a lifetime.

*Benoît Roux:* In December 2023, I used the opportunity of winning the Kenneth S. Cole Award from the Biophysical Society for my work on ion channels to express my gratitude and thank Martin for his great mentorship. I wrote that, when I joined his lab, I wanted so much to participate in the scientific enterprise of trying to understand these complex biomolecular systems, but I had so much to learn, and without him, this would have never happened. His response was so typical of his style: "Dear Benoît, Congratulations on the well-deserved 2024 Kenneth S. Cole Award from the Biophysical Society. And thank you for reminding me of the time we worked together and learned from each other. It is one of my greatest pleasure [sic] to see that one of my 'tutees' has done something special. Thank you for your kind remarks. Best, Martin." Even as a graduate student, Martin always treated us like collaborators.

*Arjan van der Vaart:* Working in Martin's groups was exhilarating; Martin had attracted incredibly talented researchers from a variety of backgrounds, he worked on a wide range of fascinating problems, and he fostered scientific discussions and the free exchange of ideas. Martin would not only provide invaluable guidance and feedback but would also offer tremendous freedom to try out new ideas and lines of research, even those not related to the original project. This approach to science has been a constant source of inspiration.

*Amedeo Caflisch:* Scientific discussions with Martin were always challenging and rewarding. He frequently asked at least one challenging question while discussing results of protein simulations, e.g., of barnase unfolding in the early 1990s. Moreover, he continuously motivated us to carefully analyze the trajectories. It was rewarding because the additional analysis almost always revealed new insights.

*Qiang Cui:* As a mentor, Martin was rather "hands off" and gave group members a lot of freedom to explore topics they found interesting once he was convinced that resources were not wasted for good reason. Martin also provided support by sending group members to relevant conferences.

*Darrin York:* Martin was an inspirational mentor whose knowledge was extraordinarily broad and remarkably deep. He fostered an environment that nurtured creativity while holding scientific contributions to the highest standards. Many of my closest friends and colleagues today emerged from the exceptional community of scientists that Martin attracted and brought together.

*Yaoqi Zhou:* My transition from simple liquids to protein folding in the Karplus group solidified my conviction that transformative discoveries emerge at the boundaries of disciplines. This mindset propelled my subsequent shifts from folding kinetics to protein structure prediction, protein design, and eventually RNA structure prediction. My tenure under Karplus's mentorship not only defined my scientific

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trajectory but also instilled the versatility to pioneer new frontiers in biophysics.

*Richard Pastor:* We were standing outside on a sunny early-spring day in the mid-1980s. Martin was visiting the NIH for a talk, and we had just finished discussing some papers from my Ph.D. that were almost ready to submit. "Do you think I was a good mentor?" I was taken aback. While Martin had always been friendly, he was reserved and had never asked me such a deeply personal question. I blurted out something along the lines that at first I didn't think that he paid enough attention to us but later realized that this was for the best and that he was a very good mentor. Can I have a do-over? I remember our conversations with great fondness, and I still strive to be a mentor as good as he was. I started working in the group in 1978, and a little while later, we met in his office to discuss my dissertation projects. He talked as he wrote on the blackboard. "You're a pretty smart guy. So first, you need to learn something about potential energy functions. How about the Lennard-Jones interactions between extended carbon atoms. Then, let's look at the difference in isomerization rates of butane from Langevin and Brownian dynamics. Finally, you said that you're interested in membranes, so do something on membranes." It has occurred to me that the "pretty smart guy" opening was just his way of getting me to agree to his list. Over the next six years, I completed the three projects, which, remarkably to me, he remembered. The first two were great learning experiences, but it was the third that shaped my career (see section lipid membranes).

*John Straub.* It was a gift to work in the group in the late 1980s, alongside Martin and so many exceptional young scientists, in our home in MB-9. I have fond memories of meetings with Martin amid the many stacks of papers in his office, our discussions of science and politics, our lively Karplus Group meetings, occasionally teaching from Martin's exceptional course notes, lunches at Harkness Commons with friends, and our Friday afternoon Karplus Group Teas.

*Matthias Buck:* Martin visited Cleveland for a conference around 2010; he said he tries to visit each of his former group members at least once to see how they were getting on. I asked him at dinner about his mentoring style. He gave the same answer he gave in "Spinach on the Ceiling" (1), where he said he provided "an environment where young scientists, once they have proved their ability, can develop their own ideas, as refined in discussions with me and aided by other members of the group. This fostered independence has been … an important element … My role has been to guide them … and to instill in them the necessity of doing things in the best possible way." When asked whether he wanted to add anything to this, he characteristically said "no"; the statement was already complete. In my own career, I have appreciated Martin's calm and rational demeanor. Most researchers in their mid-20s to mid-30s still need to grow, and working with Martin and in the environment at Harvard and at Strasbourg has been a key education for me (and I suspect for many) to adopt Martin's values as a researcher with his relentless focus on novelty and scientific accuracy.

*Rod Hubbard:* So, what was it about Martin and Harvard that was so special? We had few interactions in Harvard: our discussions were at meetings around the world or when he, Marci, and Mischa visited York. But the laboratory he and Marci fostered provided the ecosystem that allowed people like me to be innovative and explore and develop what they were good at—like the freedom I had in York. Guy and I built the York Structural Laboratory in the 1980s with a similar ethos, which I continued into the 2000s. Young researchers are given an environment in which to discover and build their own passions and profile. It doesn't work for all but is wonderfully creative for some.

*Carla Mattos:* On Martin's influence on how I do science and look at protein structure, there is no one dimension through which I can describe the influence that Martin had on the way I think and do science. There is the question-based approach, the thinking in-depth about a problem, the ethical responsibility in the way we collect and interpret data, in the way we do and communicate science. There is the real engagement, curiosity-driven desire to truly understand the world and how it works. This often requires finding the story that emerges from a sea of incomprehensible data. Simply looking at a protein structure can be a daunting task, and what I learned from Martin is to look at structure guided by purpose, letting well-defined questions find my way through atomic interactions and bringing forth hypotheses as I travel through the intricacies of the protein structure, keeping in mind the frozen instant in time that it represents. Dynamics is fundamental to how proteins work, and I also learned from Martin a deep understanding of what this means. The environment he created was exciting and productive, and those formative years have shaped how I do science and mentor my own students.

*Andrej Sali:* Martin created an environment that allowed many of us to become who we are today. He created a playground for the mind, consisting of varied cutting-edge scientific problems, tools, ideas, guidelines, and other scientists. I joined Martin's group in the Fall of 1991, to work on the development of integrative structure modeling, supported by a postdoctoral fellowship awarded expressly for this purpose. However, within the first five minutes of meeting with Martin in his office for the very first time, he suggested that I address the question of what is the difference between the folding and nonfolding protein sequences, using Monte Carlo simulations of protein folding on a lattice. Over the following three years, this clarifying question turned out to be as inspiring and productive as I ever imagined any scientific question to be. Working on the project with Martin and Eugene Shakhnovich naturally led not only to papers on the topic, but also provided lifelong lessons about science and doing science; for example, I learned about the role of

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simplified models, the need to understand as opposed to just describe, the interplay between experiment and modeling, the importance of interactions between scientists, and more. At every step of the way, Martin and the environment he created provided a guiding framework, not always explicitly, but always there. Martin remains an inspiration in my science and beyond!

*Jeremy Smith*. A few flashbacks: The humbling shock of arriving as a green 22-year-old and being assailed by all the bright Karplusians, Martin's slow, beard-stroking "okay" thought process, the ink-covered long-awaited corrected manuscripts, and, of course, afternoon foosball in the Mallinkrodt basement.

*Michael Weiss*. The scientific environment that Martin nurtured created an inspiring community of role models among peers, more senior students, and fellows, a source of encouragement and advice that was especially important for a medical student just transitioning to research. Beyond his deep insights in chemistry and chemical physics, Martin had a keen appreciation for the deep problems of biology and their medical applications. He strongly encouraged my experimental ideas in NMR spectroscopy as applied to gene regulation and later to the insulin molecule as my medical training introduced me to the growing challenges of diabetes in global health. My training was enriched by Martin's personal and professional friendships in an international community of leading scientists, including C.M. Dobson, G.G. Dodson, and A.G. Redfield.

*Wei Yang*. Working with Martin transformed my academic life. I always and still miss the moment when he dropped by my office in the morning, picked up papers I was reading, and then made comment. Later on, I saw some of these comments appear in his review articles. After many years, I started to appreciate more about how educational these conversations had been. He gave all of us freedom of exploring on our own. When he began to be involved, through many iterations of manuscript editing, we got to learn how he thought about related details. He liked to use the term "learn," and I subconsciously picked up this habit. Only after many years, when Andrej Sali talked with me and noticed, I started to realize it. I guess this was how Martin's academic gene was infused into us. Martin always appreciated my originality and was very encouraging. From him, I particularly learned how to recognize importance out of seemingly ordinary thinking and dig deep along the direction. In 2012, at Zurich, Martin told me about a topic that I should work on. I am working on it …

## CONCLUSION

The arc of Martin Karplus's scientific career originated from the founding principles of molecular quantum chemistry and ended in the modern era of the simulation of large biological systems. It is striking both in its breadth and in the fact that it followed a clear, careful path from a deep understanding of small molecules toward ever larger, biological systems. His early work, doubtless influenced by the conceptual foundations laid by his thesis advisor, Linus Pauling, developed theoretical formalisms for understanding the electronic structure and reactivity of small molecules. However, his subsequent recognition of the gap between precise quantum descriptions and the scale needed to understand biological macromolecules led him to embrace the required approximations and methods development. This shift was also at the vanguard of a conceptual realization that continues to this day to deepen: that chemistry and biology are not static but dynamic, and that understanding biological function requires exploring motion over time. The now-routine coupling of computation with crystallography, spectroscopy, and biochemical experiment stems in part from this evolution.

As this article abundantly illustrates, Karplus's laboratory at Harvard (and in Strasbourg) became a crucible for advancing the field of theoretical and computational chemistry, training generations of scientists who continue to refine and apply multiscale models in structural biology, enzymology, and drug discovery. Young researchers came in raw and questioning, sometimes unsure, and were immediately exposed to a fireworks display of theoretical ideas from other members in the group. Martin himself was rather hands off but never shirked from incisive, deep, lengthy, beard-stroking discussion with group members, and he was meticulous with manuscript editing.

Over decades, the reactions of biologists and chemists to computational chemistry have evolved in response to the efforts of the visionaries of the 1960s and 1970s. There were decades of headwinds, from, on the one side, "hard-core" theoretical chemists who saw such tools as computer graphics as nonrigorous, and from the other, experimental structural biologists dwelling on the limitations of the methods. These headwinds have now given way to general recognition that computer simulation provides deep mechanistic insights unattainable by theory or experiment themselves. Karplus's career trajectory thus reflects not only personal achievement, stemming perhaps in part from the upheaval of his experience with the Third Reich, but also was key to the broad transformation of biology into a discipline in which computation now stands as a pillar of discovery.

## ACKNOWLEDGMENTS

This article contains reflections from 43 former co-workers of Martin Karplus. The authors would like to apologize to any of the other hundreds of Karplus co-workers who may have liked to contribute but did not get a chance. This is due simply to an inability to locate them.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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