Fun and Games in Berkeley: The Early Years (1956–2013)

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Abstract

Life at Berkeley for the past 57 years involved research on the thermodynamics, kinetics, and spectroscopic properties of RNA to better understand its structures, interactions, and functions. We (myself and all the graduate students and postdocs who shared in the fun) began with dinucleoside phosphates and slowly worked our way up to megadalton-sized RNA molecular motors. We used UV absorption, circular dichroism, circular intensity differential scattering, fluorescence, NMR, and single-molecule methods. We learned a lot and had fun doing it.

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UNIVERSITY OF NEW MEXICO TO UNIVERSITY OF WISCONSIN TO YALE (1948–1956)

Most of my friends have written autobiographical articles in Annual Reviews journals—or are dead. Their chapters all relate the crucial encouragement from a Nobel Prize winner that set them on their road to success. My narrative is just the opposite; I apparently provided the spark that ignited the five Nobelists who won in the ten years after I arrived at UC Berkeley in 1956. It may have been only a coincidence, but, as I tell my students, there are no controls in life. I did, however, get valuable advice once from a future Nobel Prize winner. In 2000 at a UC Santa Cruz symposium on RNA structure, I gave my first seminar on unfolding single RNA molecules using laser tweezers. I demonstrated how beads attached to the ends of the RNA were manipulated by a micropipette and the laser trap. The beads in my demonstration were a yellow tennis ball and a yellow spider ball with black rubber hair. After the seminar, Ada Yonath, the epitome of a Jewish grandmother, advised me that the balls did not show up well against my light-colored shirt; she said I should wear a blue shirt next time. I have always needed and appreciated the guidance of my parents, wives, children, grandchildren, and students on my wardrobe and on other matters.

My arrival in Berkeley in July 1956 was all due to luck. It depended on the Korean War, my wife's unexpected pregnancy, and Stan Gill's decision to reject Berkeley's offer while Dean Kenneth Pitzer was on leave with the Atomic Energy Commission. At the University of New Mexico, my draft status was 1A and all of my classmates were being drafted for the Korean War, so I took 21 units instead of the usual 16 to finish in three years. Because I applied late for graduate school at the University of Wisconsin, all the teaching positions were filled. I accepted John Ferry's offer of a research assistantship and started research immediately in the summer of 1951. When Joan became pregnant in 1953, we decided it would be good for me to have a better-paying job before the baby came. I talked to Ferry, and he convinced John Kirkwood, Chairman at Yale, who happened to be passing by at the time, to accept me as a postdoc. Kathy was born at Grace New Haven Hospital during Hurricane Edna in September 1954, just two weeks after we arrived. Two years later, my applications for a faculty position led to only one interview trip, but the University of Kansas offered the job to future Nobelist Sherwood Rowland. Fortunately, Stan Gill turned down Berkeley's offer in favor of one from Colorado, and Jim Cason, who had been at Yale, was

Laser tweezers: a focused laser beam used to manipulate a bead attached to a molecule; the bead can apply pN forces to the molecule

acting Chairman and Dean during Pitzer's absence from Berkeley. Cason called a friend at Yale who, on Kirkwood's recommendation, talked to me in the lab. Shortly thereafter, I received a letter from Berkeley offering me a job as an Instructor in Chemistry. Our 1949 Chevrolet required a new radiator in Salt Lake City, but we made it to Berkeley in time for me to start my first, and last, academic job on July 1, 1956. I was 25.

My thesis work at Wisconsin was on the polymerization of fibrinogen, after activation by thrombin, to form fibrin, the blood clotting film. We used hexamethylene glycol to slow the polymerization enough to allow its characterization by ultracentrifugation. Biochemists tended to ignore our results because they thought experiments in organic solvents were completely meaningless for biochemical reactions. But we found that the same polymerization scheme occurred in aqueous solution, only faster. I had hoped that by now, molecular and cell biologists would have realized that experiments done in vitro or in nonbiological conditions (such as crystals) by chemists and physicists can provide them with useful information. But it is not always so. When I talk about studying the translation of one ribosome on one messenger RNA (mRNA) held between beads, someone always asks "What does this have to do with real biology? The experiments should be done in a whole cell." I agree, but I point out that single cells don't exist in isolation; the experiments should really be done in the whole organism. We'll get there eventually, as long as the National Institutes of Health (NIH) doesn't get discouraged.

At Yale, I joined a small experimental research group that Kirkwood supported. He was a theoretician, and most of his students were developing theories. We met once a week, and I was told by my labmates never to tell him a preliminary result that might be incorrect. It would be embarrassing the following week, when he had a complete theory explaining everything, and I had to tell him it was wrong. The lab project I joined sought to determine the molecular weight of egg white conalbumin. I thought that the most interesting fact about egg proteins was that they became chicken proteins. It seemed a waste to hydrolyze the egg protein to amino acids, then build it back up into a chicken protein. Why not just modify the egg protein? It is cheaper in time and money to remodel your house rather than tear it down and build a new one.

Conalbumin has a strong binding constant for iron; solutions containing it become pink from leaching iron from the glassware. Clearly, I thought, conalbumin was designed to become hemoglobin. To test this hypothesis, I incubated fertile eggs in the laboratory and analyzed the egg white proteins by electrophoresis. A beating heart and red veins soon appeared, but I saw no intermediates in the egg white that might be partially modified conalbumin on the way to becoming hemoglobin. I still am interested in protein synthesis, but I now study it at the single-molecule level rather than at the egg-chicken interface.

At the University of Wisconsin, I studied dynamic electrical birefringence of fibrinogen to measure its dipole moment and rotational diffusion coefficient (23, 29). When an electric pulse is applied to a solution between crossed polarizers, the linear birefringence produces an apparent rotation of the linearly polarized light. I wondered how the ionic environment affected the measured dipole moment and whether the circular birefringence (optical activity) of fibrinogen would change with orientation. I told Kirkwood of my interests, and he immediately discouraged my attempting to calculate effective electric fields in concentrated salt solutions—too difficult—but encouraged me to determine the effects of orientation on optical rotatory dispersion (ORD) and circular dichroism (CD). To get me started, he gave me his paper deriving the quantum mechanical equations for ORD and CD of isotropic solutions (10). My results showed that the ORD and CD did depend on the orientation of the molecule (32). In fact for helices, such as polypeptide α-helices or double-stranded nucleic acids, the CD was calculated to have a different sign for light propagating along the helix versus perpendicular to it. I showed Kirkwood my paper and asked him to be a coauthor. He said no; if his name was on the paper, people would think he had done the

Electrical birefringence: the production of different refractive indices along different directions in a solution by orienting molecules in an electric field

ORD: optical rotatory dispersion

CD: circular dichroism

research, but he hadn't. I published five papers while I was at Yale. At the time, I was proud that none had Kirkwood's name on them. Of course, now, I am sad that I didn't take the opportunity to be associated with him publicly.

The high points of my stay at Yale were the following: learning for the first time of Watson & Crick's paper (38), seeing Julie Andrews and Rex Harrison in the tryouts of *My Fair Lady* before it opened on Broadway, and babysitting Doug Rees, whose father was also a postdoc at Yale. I assume that his invitation to write this chapter was to thank me for this service.

BERKELEY (1956–2013)

On arriving at Berkeley, I presented myself to Miss Kittredge, the all-powerful administrative assistant who ran the Chemistry Department. Her first comment was, "You're very young looking." I felt like apologizing, but I don't remember what I said. Although she was now Mrs. Wilson, she had been Miss Kittredge when G. N. Lewis hired her, and so she remained so during her long tenure. My apparent youth also worried others: Dean Pitzer, now back from Washington, told me to wear a coat and tie so that I would be distinguishable from the students. Luckily, I no longer have to wear a tie or coat to be distinguishable.

OPTICAL PROPERTIES

Oriented Helices

At Berkeley, I wanted to test the theory of how ORD or CD depended on orientations of helices. The quickest way was to orient the helices by hand. We used helices made of copper wire that were 1 cm long and 0.5 cm in diameter, had 3.2 turns per cm, and were placed in 3-cm cubes of polystyrene. Their ORD and CD were measured over the wavelength range of 2.0–3.6 cm using waveguides borrowed from the microwave spectroscopists. My collaborator, Phil Freeman, was also an instructor. He made a name for himself the following year when, after presenting a controversial seminar, he pulled out a dueling pistol, laid it on the table, and asked if there were any questions. The apparatus we used is shown in **Figure 1**.

We found (30) that for right-handed helices at the 3-cm absorption band, the CD is negative for light along the helix axis and positive for light perpendicular to the axis. Right- and left-handed helices have opposite signs, and a racemic mixture (96 right-handed helices and 96 left-handed helices) has zero rotation, as does a setup with 192 empty cubes. The results agreed qualitatively with the empirical Drude equations, but we wanted to be able to relate measured magnitudes to the dimensions of the helices. We optimistically entitled the paper "1. Experimental," and our last sentence said the theory for one-dimensional conducting helices would appear subsequently. Twenty-three years later, we published the theory for helices of the same order of magnitude size as the light wavelength (17). The standard quantum mechanical theories were derived for molecules whose electrons moved short distances compared to the wavelength; a DNA helix could be 1,000 Å long, but electronic motion occurs over only a few base pairs. However, we did apply this theory to calculate the optical properties of a free electron on a small helix (35). We also measured and calculated the ORD of polybenzyl-L-glutamate α-helices oriented in an electric field, and our results were in reasonable agreement with theory (24, 25, 34).

I was pleased to learn recently that microwave determination of chirality is back in season. A new microwave spectroscopy effect applicable to polar gases at low temperatures provides absolute determination of chirality (19). The CD and ORD of a molecule depend on the product of an electric transition dipole and a magnetic transition moment. The electric transition dipole changes

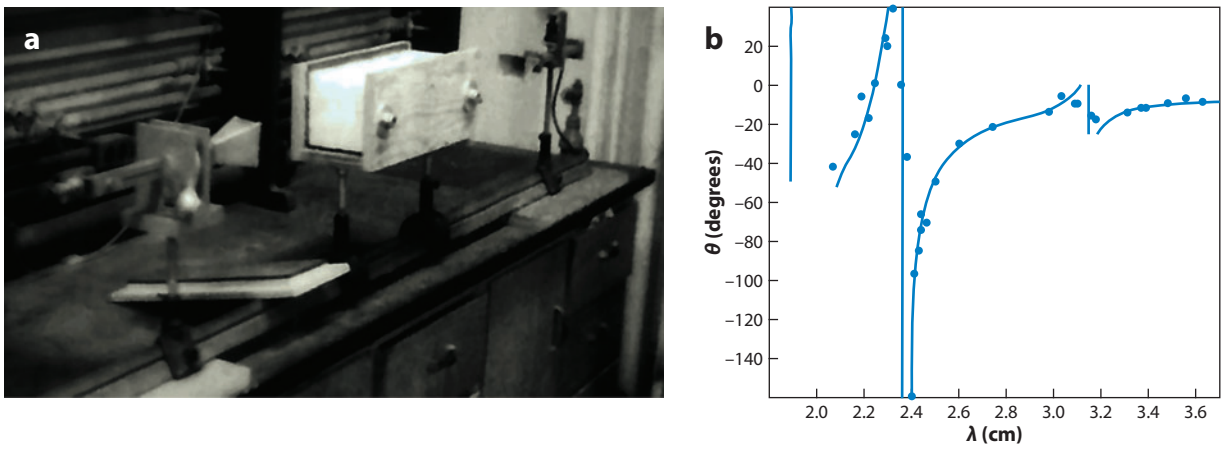

Figure 1

(*a*) An early microwave polarimeter. The polarizer (*on the right*) is a 3-cm (x band) waveguide driven by a klystron; the analyzer (*on the left*) is a 3-cm waveguide attached to a graduated wheel that could be read to less than 0.1°. Both waveguides had horns attached. The sample chamber was made of wood; it always contained 192 polystyrene cubes, which either were empty or had right- or left-handed copper helices embedded. (*b*) The optical rotatory dispersion in the microwave region of oriented right-handed helices.

sign on inversion of the wave function, but the transition magnetic moment does not, so two enantiomers have opposite signs. In the microwave experiment, the effect depends on the product of three permanent dipoles. Inversion of the wave function changes their sign, so two enantiomers have opposite signs, which can be measured by this experiment.

Hypochromicity

A troubling and puzzling fact about even pure nucleic acid solutions was that their molar extinction coefficients varied by more than 30%, depending on their preparation method, with no obvious change in their broad spectra. This was discussed widely among the physical chemists at a four-week conference in the summer of 1958 on the subject of biophysics sponsored by the NIH in Boulder, Colorado. Before I describe the science, I want to discuss the sociology of the meeting. The scientists brought their wives and children, and most families stayed the whole month. The schedule was arranged Gordon Conference style, with the afternoons and weekends free. The talks were on subjects ranging from biochemistry and molecular biology to physiology and neuroscience, and the proceedings were published in *Reviews of Modern Physics* (18). As there was no internet, people actually talked to each other in the afternoons, instead of communicating with their students, writing grants, and responding to referees. Different sports groups coalesced; I was impressed that the statistical mechanics, Norman Davidson, Terrell Hill, and Walter Stockmayer, were all excellent tennis players. Younger players, like David Davies and me, were also allowed in. I continued playing with David (on Cambridge Colleges grass courts) when we were both on sabbatical in 1964 at the Medical Research Council (MRC) lab in Cambridge (27). This meeting not only made lifelong friends among the participants, it broadened everyone's knowledge and interest over a wide field of biological science. I think it sparked new ideas, catalyzed collaborations, and actually made a significant difference in the future of biophysics. Unfortunately, an equivalent meeting is no longer possible. What junior or senior scientist would devote a month's time to learn new things not directly related to his or her research? How many spouses could leave their

Hypochromicity: the decrease in absorbance due to changes in the conformation of a molecule; often used with biopolymers such as nucleic acids and proteins

jobs for a month? Would anyone abstain from the internet for that long? Of course, the "good old days" were not so good for everyone: There were no women among the 70 invited scientists.

MCD: magnetic circular dichroism

FDCD: fluorescence-detected circular dichroism

CIDS: circular intensity differential scattering

Now back to the science. How might one explain the fact that the absorbance of the DNA depended so much on preparation conditions? I thought I could calculate the dependence of absorption coefficient on conformation using the methods previously applied to ORD and CD. Instead of calculating the dot product of the transition electric dipole and magnetic dipole operators, the square of the transition electric dipole operator was needed. The calculated result was that the ordered base transition dipoles in a B-form DNA helix had an absorbance equal to only 60% of the sum of the mononucleotides; this result agreed with experiments (26). The explanation for the large decrease in absorbance is that dipole–dipole interactions among the parallel transition moments in the stacked bases decrease the response of each base to the light. Actually the absorbance is shifted to shorter wavelengths. My paper attracted the interest of the physical chemists rather than the molecular biologists. Norman Davidson at Caltech asked Richard Feynman to present my work to his group. Feynman found that I had an incorrect term in the expression, which turned out not to significantly affect the calculated results. I proudly submitted an erratum to the journal mentioning that Feynman had found the error.

The dependence of absorbance and CD on nucleic acid or polypeptide conformation is now used routinely to follow denaturation and other changes in structure: A-, B-, or Z-form in nucleic acids, α or β structures in polypeptides. Most of the interpretation and analysis is done empirically, but I think it is important to have at least a qualitative understanding of the interactions that cause the effects.

Circular Dichroism, Magnetic Circular Dichroism, Fluorescence-Detected Circular Dichroism, Circular Intensity Differential Scattering

Research on the optical properties of biopolymers continued for many years at Berkeley. Our early instrumentation was a Beckman DU spectrometer followed by a Cary 14 recording spectrophotometer. It was a great advance when we attached a digitizer to the Cary. The output was punched tape that could be read by our Digital Equipment Corporation PDP8-S computer. The 8 meant the computer was 8 bits, and the S stood for a series (slow) interface. Shortly after we obtained the digitizer, Marc Maestre placed the trace of a previously measured spectrum back in the instrument to digitize the output by moving the pen manually over the inked line on the sheet. I happened to bring a visitor by the lab at the time; he was very impressed by how we obtained our results.

The methods investigated either theoretically, experimentally, or both included magnetic CD (MCD), fluorescence-detected CD (FDCD), two-photon CD, circular intensity differential scattering (CIDS), and differential polarization microscopy. We used FDCD to measure the CD of yeast phenylalanine transfer RNA (tRNA) from the fluorescence of the Y base in its anticodon loop (36). The FDCD result agreed reasonably well with the conventionally measured data but required only one-hundredth the concentration. FDCD thus may be useful in single-molecule detection of CD. CIDS measures the differential scattering of left- and right-handed circularly polarized light by a chiral object. The effects are large when the object is of the same order as the wavelength of the light. To test our theory, we used the helical sperm head of a Mediterranean octopus bought at a market in Barcelona by Juan Subirana. Electron microscopy showed the sperm heads to be left-handed helices with a length of about 43 μm, a pitch of 1 μm, and a diameter of 1 μm. The results of our differential scattering of 0.442-μm light showed preferential scattering of left-handed circularly polarized light, indicating a left-handed helix with a pitch of 0.65 μm, an inside diameter of 0.25 μm, and an outside diameter of 0.65 μm (14). I presented this

work at a review by the Department of Energy (DOE) of my project supported by its Health and Environmental Research division. The general topic of the grant was nucleic acid structure, so I thought the subject was interesting and not unreasonable. David Shirley, who was the director of Lawrence Berkeley Lab at that time, sat in the back row smiling throughout my presentation. He was probably wondering how he could explain octopus sperm as being relevant to the DOE mission. He never mentioned it to me, and my DOE grant was renewed.

Z-DNA and Z-RNA: unusual left-handed forms of double-stranded DNA and RNA that occur for alternating sequences of G•C base pairs

It was clear that circularly and linearly polarized light could provide specific, useful microscopic images. My colleagues invented a microscope that showed images only of oriented objects that had linear dichroism or chiral objects that showed circular dichroism (16). We applied their technology to *Drosophila* spermatocytes and to human sickle cell erythrocytes (15). The S-hemoglobin in these erythrocytes aggregates at low oxygen pressure, such as that found in capillaries, and leads to sickling of the cells. In the microscope, the linear dichroism revealed aggregation of the hemoglobin long before any changes in overall morphology of the cell appeared. We applied for a patent on the microscope, but the patent officer responded that he would not grant a patent for a microscope attached to a polarimeter. The university patent lawyer advised us that it would be easy to patent using the microscope to measure sickle cell disease, but we decided not to pursue it.

Even the conventional measurement of CD could provide surprising results. After Alex Rich at the Massachussetts Institute of Technology (MIT) discovered left-handed Z-DNA in polynucleotides of alternating guanine and cytosine deoxynucleotides (poly-dG•dC), an X-ray crystallographer explained in *Scientific American* why the corresponding helix could not form in RNA: The 2′-hydroxyl would block the structure. This was an obvious challenge, so my students tried poly-rG•rC. They found that RNA did form left-handed Z helices; it just required higher salt concentrations (6). We tried to get A-form RNA to change to B-form, but we were not able to do so; I don't know if the crystallographers have a good explanation for this result. Left-handed Z-RNA provides another example of the attitude of some biologists to supposedly nonbiological results. Before a seminar I was going to give at a university, a professor walked in and said he looked forward to hearing about my work on Z-DNA. I said Z-RNA, and he turned around and walked out. After my talk on Z-RNA at the 1984 Gordon Conference on the Physics and Physical Chemistry of Biopolymers (see **Figure 2**), someone asked me what its biological relevance was. I responded that it was of interest to at least one biological organism: me. Given the recent discovery of all types of new RNAs with surprising regulatory functions, I hope that double-stranded, left-handed Z-RNA will turn out to be the key to preventing cancer, Alzheimer's disease, or at least acne.

NUCLEAR MAGNETIC RESONANCE

NMR can be considered a continuation of our work on CD and MCD. NMR involves circularly polarized waves and magnetic fields, and it provides information on conformation. The main difference is that it uses radio frequencies, and the spectra generated have sharper lines. We started with proton NMR of dinucleoside phosphates at 360 MHz (11), and worked our way up to ^{1}H-, ^{13}C-, and ^{15}N-studies of a 56-nucleotide domain of the first ribozyme discovered, the *Tetrahymena thermophila* ribozyme (40). In between, we dabbled in studying RNA structures recommended by various people.

Olke Uhlenbeck told me that pseudoknots were the newest, most important structures found in RNA and definitely deserved study. I had heard the same lecture in the Netherlands that he had, but the significance of it had passed right over my head. A pseudoknot is not a topological knot; it is not even what nonmathematicians call a knot. In RNA, it is a structure in which the loop of one hairpin is also the stem of an adjacent hairpin. If each stem was a full turn (more

Figure 2

Our contingent at the 1984 Gordon Conference on Biopolymers. (*Top row*) Me, Ken Breslauer, Ruth Nussinov, Kathy Hall, and Doug Turner. (*Bottom row*) Phil Borer, Sue Freier, Olke Uhlenbeck, and Art Pardi.

than 11 base pairs), it could be considered a knot. After our publication of a small nonbiological RNA that could form a pseudoknot (20), Harold Varmus (then at the University of California, San Francisco) called me and said that he would be interested in the structure of a pseudoknot critical for causing frameshifting in the mouse mammary tumor virus mRNA. Retroviruses use frameshifting to synthesize a polyprotein that contains enzymes vital for the propagation of the virus: reverse transcriptase, integrase, and protease. The ratio of these proteins is critical, and the process of synthesizing them is called a programmed frameshift. The protease cuts the polyprotein to release the reverse transcriptase, which synthesizes the viral DNA from the RNA, and the integrase, which inserts the viral DNA into the host genome. It is efficient for the viral mRNA to hold on to a host ribosome after synthesizing a protein. Instead of letting go and competing for another ribosome, the mRNA induces a frameshift and makes a new protein attached to the old one. Varmus hoped that the structure of the pseudoknot, as well as that of mutant mRNAs with reduced frameshifting ability, might explain the biological mechanism underlying frameshifting. Unfortunately, our published structural results did not provide a conclusive explanation (9, 22). Presumably, further work that includes the interaction of the pseudoknot with the ribosome will eventually lead to an answer. High-resolution structures of mRNAs with and without wild-type and mutant pseudoknots may help, but a dynamic motion picture of the movement of the mRNA through the ribosome (determined by single-molecule methods) will probably be necessary.

Larry Gold wondered why an RNA hairpin with the loop sequence UUCG blocked the passage of the reverse transcriptase used in sequencing RNA. We found that the loop has an unusual *syn* guanine conformation, rather than the typical *anti* conformation; it forms a G•U base pair across the loop (4). This extrastable UUCG loop may serve both as an initiation site for RNA folding and as a specific protein binding site. Its structure distinguishes it from the 255 other possible tetraloops in RNA and provides a logical explanation for its biological effects. The corresponding DNA tetraloop has no unusual structural interactions and presumably no special function (7).

We also investigated other RNA structures including various loops, bulges, mismatches, triple helices, and kissing hairpins, among others. Some of the sequences had biological functions; others did not. The main result was to provide examples of structures that could form in RNA.

BULK THERMODYNAMICS AND KINETICS

In the early days, our main source of RNA samples was hydrolyzed yeast RNA. Chromatography provided mixtures of RNA oligonucleotide dimers, trimers, and larger fragments that could be further purified. We first measured the absorption and ORD of the 16 dinucleoside phosphates (37), after which we studied 7 of the 64 trinucleoside diphosphates (3). This work led us to three conclusions: The optical properties of the trimers can be approximated reasonably well from the monomer and dimer spectra; adenine bases stack significantly more than do uracil bases; and measuring the properties of all 64 trimers and all 256 tetramers is probably not necessary to understand single-strand RNA spectra.

The correlation of the dimer-trimer spectra led to the idea that the stabilities of double-stranded RNA might be sums of the stabilities of its subunits. Olke Uhlenbeck came to Berkeley with double-stranded oligonucleotides from his thesis project. More importantly, he brought a polynucleotide phosphorylase that could be used to synthesize other oligos. We started measuring absorbance melting curves of double-stranded oligos. The easiest to study and analyze were self-complementary oligos, such as A_NU_N. We called these "69" duplexes.

The first attempt to predict secondary structure in RNA and melting temperatures of duplex RNA was published in 1971 (33). We assumed that RNA folding was determined by thermodynamics, not kinetics; therefore, the actual secondary structure would minimize the Gibbs free energy value. However, we arrogantly thought that biochemists would be confused by being told that more negative values meant more stable structures, so we defined stability numbers. Each G•C base pair contributed +2, an A•U pair contributed +1, and G•U contributed 0. Unpaired loops and bulges contributed negative stability numbers depending on how many unpaired nucleotides they had. Possible base-paired structures were considered, and the one with the largest stability number was the correct one. In the next iteration, we acknowledged that biochemists, or at least the newly christened molecular biologists, could understand free energies (28). We used Gibbs free energies with the contributing subunits being two base pairs, not one. This is the nearest-neighbor approximation; the free energies of base-paired regions are the sum of the 10 Watson–Crick nearest-neighbor base pairs. The values for these parameters were obtained by measuring the melting curves for oligonucleotides and polynucleotides (2). We used 1 M NaCl as the solvent because we wanted to reduce the electrostatic repulsion between the oligonucleotides and because heating RNA solutions in Mg^{2+} would lead to hydrolysis. Obviously, 1 M NaCl is not very physiological; potassium glutamate plus Mg^{2+} might be much better. However, we did find that although replacing some of the Na^+ with Mg^{2+} affected tertiary interactions, it had a much smaller effect on double-strand formation. The research has since been extended by others in many ways; free energy values have been obtained for G•U nearest neighbors, hairpin loops, internal loops, bulges, dangling ends, and other structures. I am amused that Doug Turner, who had never heard of Lewis and Randall before he came to Berkeley, is now the world's expert on thermodynamics of RNA.

SINGLE-MOLECULE THERMODYNAMICS AND KINETICS

In 1998, Carlos Bustamante, who had received his Ph. D. at Berkeley in 1981, returned to campus as a faculty member, bringing with him the pioneering designer and developer of laser tweezers,

Figure 3

The RNA warriors: Jan, me, Princess Bibiana, Carlos, and Steve.

Steve Smith. They had used laser tweezers to measure the mechanical properties of DNA, and it was obvious that RNA, with its ability to form a wide range of secondary and tertiary structures, would be a much more interesting subject. Bibiana Onoa had applied for a postdoc with Carlos to work on atomic force microscopy, and Jan Liphardt had applied to work with me on NMR of pseudoknots. Carlos and I convinced them to do single-molecule studies instead, specifically to measure the thermodynamics and kinetics of unfolding RNA. To me, the idea that one could study a single molecule in solution must involve magic or at least science of the future as it is described in science fiction. However, I suspended my disbelief and helped convince the two new postdocs that they had a reasonable project (see **Figure 3**).

An RNA hairpin was held between two micron-sized beads; one bead was in a laser trap, and the other was on the end of a micropipette. The distance (in nm) between the beads was measured using a light lever; the force (in pN) was determined by the bead's position in the trap relative to the center of it. The focused laser beam forms a harmonic well that acts as a spring attached to the bead. By measuring the force and distance, we measured the work (equal to the force times the distance) for unfolding the stem-loop of the RNA hairpin to a single strand. At constant temperature and pressure, the reversible work is equal to the Gibbs free energy change.

However, often the kinetics of a reversible reaction are so slow that their direct measurement is impractical.

As our first experiment, we chose a simple RNA hairpin of 22 base pairs in 10 mM Mg^{2+} at 25°C (13). As we were unfolding by force, not temperature, we could use Mg^{2+}. We increased the force on the ends of the hairpin until it unfolded to a single strand, which was then stretched to near its contour length. At a force in the range of 14–15 pN, the molecule hopped back and forth between closed and open forms. The ratio of the time spent as a hairpin to time spent as a single strand is the equilibrium constant for the transition. The logarithm of the equilibrium constant was linear in force—analogous to the dependence of ln K on temperature ($1/T$). These experiments were used to obtain the equilibrium constant at zero force and the standard Gibbs free energy change for unfolding the hairpin. The results agreed with the value obtained by thermally melting the hairpin using classical ensemble thermodynamics. This was rewarding because the classical thermodynamicists wondered if it made sense to apply thermodynamics to a single molecule. We pointed out that as our single molecule was in a bath of $\sim 10^{16}$ water molecules, our system actually included many molecules. However, I am convinced that even a single gaseous molecule in a vacuum can be treated usefully by thermodynamics. Unfortunately, the time to attain equilibrium will be very slow.

Repeated measurements of work under irreversible conditions can be used to obtain the reversible work and thus the Gibbs free energy. Christopher Jarzynski proved that the average of the Boltzmann-weighted irreversible work values, w, gave the reversible work, the Gibbs free energy change, $\triangle$G (8).

$$e^{-\Delta G/k_B T} = \left\langle e^{-w/k_B T} \right\rangle.$$

This result was surprising to many thermodynamicists; they weren't even convinced that it was correct. Although the Second Law states that the free energy increase in a system is equal to or less than the average work done, people tend to forget the word average. Fluctuations can occur in the process, adding more work (at constant T and P) than the amount that corresponds to the free energy increase. The Jarzynski equation is exact for an infinite number of values measured, but the number of repetitions required to give a useful result depends on how far from reversible the process is. The equation requires that some of the measured irreversible work values be greater than the measured reversible work values. The probability of this occurring is very small when the system is far from equilibrium, meaning that an impractical number of repetitions of the experiment would be required to obtain a useful free energy. By varying the rate at which we increased the force used to unfold the RNA, we were able to vary the irreversibility of the reaction and to test the number of experiments needed to get good results (12).

Unfolding RNA with tweezers gave useful new results, but this technique was not likely to lead us to completely unexpected places. However, studying the translation of one mRNA by one ribosome might. A chemical reaction quickly loses synchrony because of the stochastic nature of kinetics. It is thus impossible to follow more than two or three translation steps in a bulk translation experiment. However, in a single-molecule experiment, each step in a reaction can be seen. Processes obscured by averaging over many steps will become visible. By holding a hairpin between two beads at a constant force below that required to open the hairpin, we could watch the hairpin open as the ribosome translated the mRNA. For every three-nucleotide codon translated, there would be a six-nucleotide increase in the length of a single strand. Of course, the thought experiment is much easier to do than the real one. Several postdocs and graduate students worked hard on the project before Jin-Der Wen succeeded (39). Raven Hanna and Ana Zeri helped in the effort. Raven now lives in Hawaii, where she manufactures beautiful jewelry containing molecular

structures; her website is **http://store.madewithmolecules.com/**. Ana returned to NMR research in her home country at the Brazilian National Biosciences Laboratory.

FRET: fluorescence resonance energy transfer

Jin-Der's main change in technique was to use purified translation cofactors rather than the S100 mixture of all soluble *Escherichia coli* proteins traditionally used by in vitro translators. He saw pause-step-pause trajectories with seconds-long pauses and milliseconds-long translocation steps. Each translocation step released six nucleotides as expected. The ribosome spends 1–2 s stopped at each codon while it waits for one elongation factor, EF-Tu, to bring in the next amino acid and a second, EF-G, to catalyze translocation. Then, the ribosome quickly jumps to the next codon. The distribution of dwell times at each codon shows that more than one reaction contributes to the dwell time. The kinetics fit a Michaelis–Menten mechanism (21): Decreasing the concentration of EF-Tu or EF-G changes the dwell-time distribution to correspond to a single rate-determining step.

I am convinced that learning and teaching single-molecule methods are great ways to understand a difficult and nonintuitive topic in physical chemistry, such as thermodynamics. In single-molecule experiments, one can see the reactions occurring in a solution at equilibrium as a molecule hops back and forth between two species. Similarly, a better understanding of kinetics is obtained when one considers the reactions of single molecules. Single-molecule fluorescence resonance energy transfer (FRET) can also provide unique information about complex multistep processes such as translation (31). Obviously, combining force and FRET will be even more powerful (5).

FUN AND GAMES

I tell new students when they join the group that their duties are to entertain me and to educate me, in that order. Each has done so in various ways and to various extents. (I want to insert a personal message here to each of my students, "You are the best student I have ever had.") Of course, I hope that they will also be entertained and educated. Graduate school is not always fun; experiments don't work, or they do work but give an answer the boss doesn't want to hear. Research directors expect instant results, not remembering or caring that you are teaching, maybe raising a family, and trying to lead a life. A student told me that an appropriate epitaph for my tombstone would be "That shouldn't be too hard to do." She obviously hoped that the tombstone would be needed sooner rather than later.

I believe that if science is not fun, you shouldn't waste your life doing it. Obviously watching chromatography columns is fun. But outdoor exercise is also good for you, and fun. These facts are all illustrated in **Figure 4**.

We liked to bet on the melting temperature of each new double-stranded oligonucleotide that was synthesized. As serious sums (quarters) depended on the measured values, everybody in the lab was eager to see the result and to be sure the measurement was done correctly. These bets increased the rapidity and validity of the data. We also did indoor physical exercise. When Arlene Blum broke her leg skiing, the student health service lent her an old wooden wheelchair. The students of the groups on the third floor of Hildebrand Hall (Hearst, Tinoco, and Wang) raced around the corridor in the wheelchair, until one of them broke a finger caught between one of

Figure 4

(*a*) Me, proclaiming the joy of chemistry while (*b*) Carol Cech and Sue Freier are experiencing it. (*c*) Olke Uhlenbeck and Fritz Allen praying over a column. (*d*) Friday afternoon at Indian Rock in Berkeley: Marty Itzkowitz, Curt Jonson, my daughter Kathy, me, Sunil Podder.


a
the JOY of CHEMISTRY
b
c
d

the spokes and the armrest. We also competed in great ideas contests (indoor mental exercise). Some students refused to participate; they didn't want to be judged by their peers or research directors. I would sometimes put a number series on the board, so people could try to deduce the next number (this was before Google existed). Some would not try, in case they failed or because they felt that it might take them longer to see the answer than others. I think scientists should be willing to attack any problem, should not be afraid to fail, and should not worry about how long it will take them to solve the problem. In science, the important thing is to find the right answer; it doesn't matter how many stupid mistakes you make along the way. Norbert Wiener, an MIT mathematician, said that in chess, the first one to make a mistake loses; in science, however, the first one to find the answer wins.

This makes me think of a science fiction story, "Transstar" by Raymond E. Banks (1). In the story, the job of the Transstar foundation was to keep the human race on planets around other stars safe from aliens. The foundation was always being asked for help, but it responded that the interactions between the colonists and aliens were only a local problem, not a Transstar problem. If the colonists couldn't handle those interactions themselves, maybe they should move to another habitable planet; there were plenty for everyone. But on one planet, the aliens started using the human females to breed pets and dimwitted slaves, claiming that there was not enough room in the universe for humans and themselves. This was definitely a Transstar problem. The complete manufacturing capability of the solar system was dedicated to building the necessary spaceships, and all eligible people were drafted. The humans defeated the aliens so overwhelmingly that the alien leader said that there was plenty of room in the universe, and they would stay out of the human sectors in the future. I Xeroxed the story, wrote on it, "Is DNA sequencing a Transstar problem?", and gave it to my students. We discussed possible attacks on DNA sequencing but did not pursue any of them. However, I did end up as chairman of the DOE committee that first proposed a massive effort to sequence a human genome in our "Report on the Human Genome Initiative for the Office of Health and Environmental Research, April, 1987." We recommended that a total of one billion dollars in DOE funding would produce the complete sequence of at least one human chromosome, as well as allow the United States to sequence ten million bases per day by 1995. The proposal was not popular with most molecular biologists. They did not admire much of the DOE biological research and were worried that a Manhattan Project–style investigation would steal money from them. However, our prediction was not too far off. Our proposal energized the NIH to quickly become the dominant partner, and the Human Genome Project was officially recognized as finished by 2001.

It is obvious that you should choose important projects and devote your full effort to them. Linus Pauling's advice, which I heard as an undergraduate at the University of New Mexico, was that you should become the world's expert in some field. Assuming it was an important field, you would be invited to conferences all over the world to present your work. He did not say it, but I thought that even if the field was not too important, you would have the satisfaction of knowing that you knew more about this field than anyone else, even if nobody cared. I think the best scenario is to pick a fun problem that you think you can solve, then learn that it is important.

My group members didn't spend all their time at Berkeley; sometimes we even left California, mainly to show the flag. **Figure 5** shows the RNA flag on the summit of Mt. Waddington, placed there by Arlene Blum. The flag also made it to Yosemite, but it was apparently forgotten at Artist's Palette in Death Valley.

Cold Noodles

If there is one piece of advice I can give to the scientific community, it is "cold noodles." This is the traditional meal we have at our parties; I can recommend it very strongly for groups of any size

Figure 5

(*a*) RNA flag on the summit of Mt. Waddington, Canada. (*b*) Dave Lloyd, me, Marty Itzkowitz, Curt Johnson, and Barrett Tomlinson on Artist's Palette in Death Valley, CA. (*c*) RNA flag over me, Kyong Yoon, and Adrienne Drobnies in Yosemite.

between 10 and 100 people. The dish consists of Chinese noodles with side toppings of chicken, cucumbers, green onions, carrots (all sliced thin), and bean sprouts. The unique ingredient is the peanut butter sauce, which contains garlic, ginger, sugar, soy sauce, vinegar, red wine, and hot sauce to taste. The participants construct their own plates, so vegetarians are protected. The food is delicious if the recipe handed down from David Koh to Barbara Dengler to Bibiana Onoa is used for the sauce. Barbara Dengler (1967–2013) and David Koh (1968–2000) have kept the lab running forever and still provide cold noodles.

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