# 6.1: Prelude to Organic Reactivity

September 5, 1966, turned out to be a very good day for Hudson Freeze. An undergraduate microbiology major at Indiana University, he was a few weeks away from the first day of classes in his junior year, but on this September day he was far away from the oppressive heat and humidity of late summer in the midwest. Instead, he was working at the edge of Mushroom Spring in Yellowstone National Park, one of one of the many geothermal hot springs for which the park is so famous.

At the end of his sophomore year, Freeze had approached Dr. Thomas Brock, one of his microbiology professors, to ask about the possibility of working as a research assistant over the summer. Brock took him up on the offer, inviting him to come out to Yellowstone for a few weeks in late summer to help with some fieldwork.

For the past few years, Brock had been studying microbes that inhabited the hot springs: these 'extremophile' organisms were fascinating to him because they appeared to thrive in conditions that until quite recently had been thought to be too hot to support life. The currently accepted upper limit at which life was believed to be possible was 73 ºC, but during his work in Yellowstone the previous summer Brock was convinced that he had observed microbial life – a pink colored, filamentous bacteria - in water as hot as 88 ºC. Unfortunately, all of his attempts to culture these life forms in the lab had been unsuccessful. He had decided to focus his efforts this summer on Mushroom Spring, where the water was 73 ºC, right at the supposed limit for life, and assigned Freeze the task of collecting microbial samples from the waters of the spring. On September 5, Freeze collected a promising-looking sample, which he took back to a makeshift lab in Brock's cabin to prepare for transport back to Indiana.

A few weeks later, working in his professor's lab in between classes and homework, Freeze was engaged in the challenge of figuring out how to get his microbes to grow outside their natural environment, so that he could isolated and eventually characterize them. The work was frustrating at first – attempting to get the bacterial to grow in a liquid medium, he never observed the characteristic turbidity that usually signals success. In some samples, however, he did observe the appearance of salt crystals on the bottom of the test tubes. He allowed these samples to incubate for a few more days, and noticed that more crystals had formed. Just to be thorough, he decided to look at some of the crystals under a microscope – and hit the jackpot. Clinging to the crystals themselves were the recognizable shapes of microbial cells.

In subsequent work with Thomas Brock, Freeze was able to improve his culturing techniques and characterize the new species of bacterium, which was later named *Thermus aquaticus*, or 'Taq' for short. He also was able to demonstrate that enzymes isolated from the bacterium remained intact and active even in boiling water.

Even though Yellowstone is a beautiful place to spend a few weeks in the summer doing field work, it turned out that making the trip to Wyoming was not really necessary – Thomas Brock later was able to isolate cultures of Taq from samples taken from the hot water system right there on the Indiana University campus, as well as from many other hot-water environments around the world. Brock and Freeze went on to publish a paper in the Journal of Bacteriology (1969, 98, 289) describing their newly discovered species, and donated live cultures of Taq to the American Type Culture Collection, a biological repository in Washington D.C.

Years later, a scientist named Kary Mullis working at Cetus, a biotechnology firm in the San Francisco Bay area, purchased a culture of Taq - a direct descendent of the very culture that Hudson Freeze had taken from Mushroom Spring on September 5, 1966 - from the ATCC repository. Cetus paid $35 for the sample. It turned out to be a pretty good investment.

Mullis and his colleagues at Cetus were intrigued by Freeze's report years earlier that enzymes isolated from Taq were stable at high temperatures, unlike enzymes isolated from E. coli and other common model organisms. They cultured their Taq sample, purified the DNA-copying enzyme DNA polymerase from the Taq cells, and using the heat-stable polymerase were able to come up with a remarkably efficient method for copying short stretches of DNA. Their 'polymerase chain reaction', or PCR, went on to revolutionize the fields of molecular/cellular biology and biochemistry – read through the experimental section of any recent research paper in one of these fields and chances are you will see that the researchers used PCR. If you take a lab course in molecular biology, you will probably perform at least one PCR procedure. When your professor purchases the purified Taq polymerase enzyme and other reagents for your lab, part of the price will go towards paying royalty fees to the pharmaceutical giant Hoffmann-LaRoche: Kary Mullis and Cetus obtained a patent for their PCR process, and in 1992 sold patent rights to Hoffmann-LaRoche for $300 million. Mullis was awarded the 1993 Nobel Prize in Chemistry for his work on PCR.

What makes the PCR technique so powerful is that it harnesses a biological catalyst - the DNA polymerase enzyme naturally produced by the Taq microbe - to vastly increase the rate of a very specific and very useful chemical reaction, under environmental conditions (high temperature) that until then had been fatal for other enzymes. Taq polymerase, the $300 million molecule, is the most visible example (for now!) of how scientists might harness the power of biological catalysis to great advantage, but many researchers are hard at work, in Yellowstone and many other locations around the world, writing more chapters in the story that was begun by Hudson Freeze and Thomas Brock on a September day in 1966.

Up to this point, we have been focusing on the structure of organic molecules: essentially, how these molecule are put together. Now our focus shifts to the study of reactivity: what happens, in other words, when covalent bonds within a molecule break and new covalent bonds form, as molecule A is transformed into molecule B. The story of Taq and PCR is centered around a biochemical reaction - the polymerization of DNA starting with an existing DNA 'template'. We are about to begin our exploration of chemical reactivity: how a reaction is depicted on paper, whether it absorbs or releases energy, how fast it goes, and how a catalyst might be able to make it go much faster.

In your previous college general chemistry and high school chemistry courses (and perhaps in biology courses as well), you have no doubt seen many different examples of chemical reactions. Most likely, these reactions were depicted by chemical equations, showing the starting materials (reactants) and the finished products connected by a 'reaction arrow'. In most cases, the structures of reactants and products were not considered - they were defined only by molecular formula and perhaps by physical state (solid, liquid, gas, or solution). The reaction below, showing the decomposition of dinitrogen pentoxide ($N_2O_5$) to nitrogen dioxide and oxygen, is a typical example of the 'equation' treatment of chemical reactivity which you might have seen in your General Chemistry textbook.

$$N_2O_5(s) \longrightarrow 2\,NO_2(g) + \tfrac{1}{2}O_2(g) \tag{6.1.1}$$

This way of talking about chemical reactions is perfectly adequate in introductory chemistry classes, when fundamental chemical concepts like stoichiometry, thermodynamics, and kinetics are being explained for the first time. In organic chemistry, beginning with this chapter, we will go much further. We will certainly review the important fundamental concepts of thermodynamics and kinetics that you learned previously. But in our discussion of organic reactivity, we will bring our understanding of organic structure into the picture, and think about how reactions take place: which bonds break, which bonds form, why a particular bond breaks or forms, the order in which bond-breaking and bond-forming takes place, and the nature of any intermediate species that might form during the course of the reaction. We also will think about how catalysts - enzymes in particular - are able to increase the rate of a particular reaction. Taken together, a description of a chemical reaction at this level is called a reaction mechanism. Beginning here, and continuing throughout the rest of the text, our main job will be to understand the mechanisms of the most important types of reactions undergone by organic molecules in living organisms.

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