

**Eigen M.** Proton transfer, acid-base catalysis, and enzymatic hydrolysis. Part I: elementary processes. *Angew. Chem.* 75:489, 1963; *Angew. Chem. Int. Ed.* 3:1-19, 1964; and Selforganization of matter and the evolution of biological macromolecules. *Naturwissenschaften* 58:465-523, 1971.

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1963: The development of relaxation techniques made possible a quantitative study of fast elementary reactions and a dissection of complex reaction mechanisms, which could be explained in terms of elementary steps. Application to biological reactions led to the conclusion that enzymes are optimal catalysts. [The *SCI*® indicates that this paper has been cited in the 1963 form in over 285 publications and in the international edition (1964) in over 965 publications.]

1971: Biological self-organization is based on natural selection. A prerequisite of natural selection is self- or complementary-reproduction. Such a process generates information, which is the physical basis of life. [The *SCI*® indicates that the *Naturwissenschaften* paper has been cited in over 490 publications, making it the most-cited paper for this journal.]

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## Enzymes Are Optimal Catalysts

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The two *Citation Classics*—both written within an interval of seven years—seem, at first sight, entirely unrelated. This impression, however, is superficial. There is, indeed, a strong internal tie that becomes obvious if we analyse the objective and subjective motives that led to the writing of these articles.

During the 1950s most of our work concerned the development of relaxation techniques for studying fast reactions in solution.<sup>1</sup> Leo DeMaeyer, Joseph Schoen, Gerhard

Schwarz, Georg Czerlinski, Hartmut Diebler, Walter Kruse, Günter Maass, and Georg Ilgenfritz—among others—contributed much to this early technologically oriented phase of work. Waves of visitors, students, postdocs, and professors (on sabbatical leave), who came from all over the world, swept through our laboratory. I remember that it was always crowded. We had a high turnover of both staff and machines because many coworkers took home either a secretary or a T-jump device when they left. The first wave of visitors consisted mostly of inorganic chemists. They were followed by the organic chemists who eventually mixed with the biochemists. Today the center of gravity has shifted to what we call molecular biology (including mathematics, physics, chemistry, microbiology, genetics, and even electrotechnics).

After it had become possible, using the methods of relaxation spectrometry, to dissect complex reactions into their elementary steps, and thereby measure the rates of very fast processes such as the transfer of protons through hydrogen bonds, it was merely a matter of a few years before these new tools were applied to biochemical processes and enzyme mechanisms were elucidated in every molecular detail.<sup>2</sup> It was not at all surprising to find that these molecular gadgets, whose biological origin is a notorious problem, obey the laws of physics and chemistry. Wherever a violation of these laws seemed to occur, detailed analysis uncovered nature's tricks. An example is pertinent: The maximum speed of a bimolecular reaction is limited by the encounter rate based on the diffusional motion of both reaction partners. However, for the binding of the lac-repressor to its DNA operator site, a value exceeding the maximum rate constant for a diffusion controlled process by one to two orders of magnitude was obtained (depending on reaction conditions such as ionic strength, etc.). It turned out that neither theory was wrong nor was there any demon at work. The repressor is able to bind at any DNA site via electrostatic interaction after which it is guided through one-dimensional diffusion along the helix-axis into the operator site.<sup>3</sup> Other studies even revealed "intelligent" reaction behaviour of enzymes. With the new tech-

niques, we were able for the first time to break down an allosteric mechanism into its single steps.<sup>4</sup> Jacques Monod was delighted to find his cooperative model of allostereism fully confirmed.<sup>1</sup>

Our conclusion that "enzymes are optimal catalysts" is to be understood in this sense: they are best adapted, given a number of sometimes counteracting constraints. Optimal efficiency does not plainly mean maximum physical speed; it may involve an optimal compromise between specificity, speed, stability, and other chemical constraints, and in some cases it may involve "intelligent" control of rate and specificity.

If all this is true, we are forced to ask: Who constructed those optimal gadgets? The biologist will answer: natural selection! But what does this mean, if molecules rather than living entities are involved? What is the target of selection? Molecular structures usually prevail because they represent states of minimum potential energy. Why should such a property coincide with optimal functional performance?

Is biological organization at the molecular level at all a physical problem? Isn't optimal efficiency of enzymes rather the result of chance and therefore unique and not reproducible? Or are there physical principles, similar to the extremum principles of thermodynamics, that guarantee optimal solutions?

It was clear from the beginning that the solution of the problem had to be of a Darwinian type, although Darwin had never dealt with molecules. It turned out that no equilibrium theory of molecular organization could do the job and that one of the principle requirements of selection and evolution is self-reproduction. The prerequisite is fulfilled by all living crea-

tures, but on the molecular level only nucleic acids inherit this ability. (My late friend Sol Spiegelman used to say: Man is only a trick of nucleic acids to reproduce even under strange conditions, for instance, on the moon). However, the theory was not just an adoption of Darwin's tenets and their application to molecules. What finally resulted was a picture of a much more subtle nature: Selection at the molecular level appears to be a "phase transition" in information space.

Information melts away whenever an error threshold—well defined by the theory—is exceeded. Notice that it is not matter that melts away. Rather it is information that represents functional meaning. This property is as immaterial as Mozart's music, which also has to be fixed on (material) scores. Information is a property that transcends the material character of chemistry and distinguishes a living organism from any chemical composite, no matter how complex.

Sequence space, fitness landscapes, quasi-species, and hypercycles are abstract concepts that have been developed in order to *understand* complex reality rather than to describe or represent it.<sup>5,6</sup> Meanwhile these concepts have been tested experimentally and are the basis of a new evolutionary biotechnology.<sup>7</sup> At the same time, sequence space, error threshold, and quasi-species are concepts that are reflected most directly in the reality of viral life.<sup>8-11</sup>

Peter Schuster, now at the University of Vienna, was an early partisan in the development of all these ideas.<sup>5</sup> He, and later John McCaskill and others, contributed many original thoughts to what now appears to be a well-established edifice.<sup>6</sup>

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