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THE CONCEALED SIDE OF THE HISTORY OF COMBINATORIAL CHEMISTRY

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Abstract

Combinatorial chemistry today is an accepted new branch of science that comprises methods widely applied in drug research and other research fields within chemistry and even outside of it. Its beginnings were slow and painful in the 1980s. The present account describes a story that has not been heretofore revealed in the history of its discovery. Its format is a conversation in which Árpád Furka, the original discoverer, provides the answers to István Hargittai's questions.

Keywords: combinatorial chemistry, split-mix method, peptide synthesis, science history.

How and when did you come to idea of the combinatorial method?

Around 1980, I began to think about the possibility of preparing all members of families of peptides: dipeptides, tripeptides, tetrapeptides, or pentapeptides. It was clear from the beginning, that this could be accomplished only by a new method since a very simple calculation showed that using the available techniques, thousands of years would be needed to synthesize, for example, all the pentapeptides. Considering the possible solutions, it was also clear that multi-component mixtures of peptides could easily be prepared by using mixtures of amino acids – instead of the usually applied single ones – in their solid phase synthesis.

On the other hand, this did not seem to be an acceptable solution because of the differences in the reactivity of the activated amino acids that would lead to the formation of peptides in significantly different concentrations, thus causing problems in screening. The goal was to eliminate the problem stemming from reactivity differences. I solved the problem in 1982, working in Budapest.

My idea was to make the couplings with single amino acids on equal samples of the resin, then mix the samples after coupling. This made it possible to force the coupling reactions to completion with each amino acid and thus assuring the equal molar formation of peptides. This is the simple and well-known split-mix

combinatorial synthetic method that reduced the time needed to prepare the peptide libraries from thousands of years to a few days.

Did you have any idea of how to utilize the peptide mixtures?

Finding the bioactive peptides in a mixture of millions of components seemed like finding a needle in a haystack. So a strategy was needed to solve this problem and this strategy (later known as the iteration method) was also ready in 1982.

Did you try to find applications for the idea?

I discussed the problem of making a patent with a patent attorney Dr. Éva Somfai with whom we worked together in filing other patents for a Budapest pharmaceutical company. It turned out that patenting would cost more than I could afford. The pharmaceutical companies were not interested in patenting our method. As a safeguard, she suggested to describe the method in a document and notarize it. This could help in the future in possible priority disputes. I did so and the document was notarized on June 15, 1982. The document can be seen in both the original Hungarian and its English version in my home page [1] and it was also published in *Drug Discovery today* [2].

When did you first publish the method?

When I discussed the new synthetic method with colleagues I experienced a strong disbelief. This was the reason why I thought for a long time about finding the best way of publishing. Finally we published the synthetic method on posters at two international congresses in 1988: The 14th International Congress of Biochemistry, Prague [3] and The 10th International Symposium of Medicinal Chemistry, Budapest [4]. Although no particular interest or response was forthcoming, in hindsight it was most fortunate that at least these Abstracts about the initiation of combinatorial chemistry had gone on record. However, the lack of responses by chemists and my failure to find cooperating biologists quite disappointed me. It took a while before I decided to submit a manuscript for a full paper.

What was your choice then for your publication?

It was clear to me that it would be impossible to publish the method in a general journal like *Nature*. So I chose a more specialized periodical, the *International Journal of Peptide and Protein Research* in which we had published before although we knew that the Chief Editor of the journal had changed since that time.

In our experiments we focused on proving that the expected peptides do form in the synthetic procedure. For this reason only small libraries could be used. We developed a computer-aided paper electrophoretic procedure [5] that

was successfully used in identifying the components of the synthetic mixtures. The manuscript, entitled 'General method for rapid synthesis of multi-component peptide mixtures' was sent to Professor Hruby, University of Arizona, Tucson, and was received on February 12, 1990. The manuscript was reviewed by three reviewers. One of them, although accepted the paper with minor revisions, wrote in his comments: "Having spent years endeavoring to prepare peptides in the pure state, manuscripts like this 'compromising with mixtures' (p.3) cause me some anxiety." In his letter of May 15, 1990, Professor Hruby wrote that the paper may be acceptable only after major revision. After doing further peptide separations using HPLC, the revised version of the paper was accepted on November 21, 1990, and appeared in print in June 1991 [6].

I have heard rumors that there were some disappointing events connected with your publication. Could you tell us what these events were?

Our paper appeared 16 months after submitting the manuscript. During this period, patent applications were submitted by four different groups all based on our split-mix method: HUEBNER and SANTI (Chiron group) [7] on May 15, 1990, LAM et al. (Salmon-Hruby group) [8] on July 2, 1990, HOUGHTEN et al. (Richard Houghten's group) [9] on November 21, 1990 and DIMARCHI et al. (Eli Lilly group) [10] on June 18, 1991. It was particularly painful that Professor Hruby, the Editor-in-Chief of the journal where our manuscript was submitted, participated in patenting and his group also submitted a paper to *Nature* [11] and presented a lecture to the 12^{h} American Peptide Symposium [12], claiming in both papers that they invented the split-mix method. Shortly after the appearance of our paper, Dr. Richard Houghten and his group (Torrey Pines Institute for Molecular Studies, San Diego) made also a presentation at the Symposium on the Innovation & Perspectives in Solid Phase Synthesis and Related Technologies [13] and submitted a paper to *Nature* [14] in which our split-mix synthesis was described. I would also like to call your attention to a remarkable coincidence. The number of the filed patents was exactly the same as the number of those that had had access to our manuscript: the editor of the journal and three reviewers. Do you think that such a radically new idea like that of the split-mix synthesis may suddenly and independently occur in four different heads not earlier and not later but exactly at the same time when our manuscript is being reviewed for publication, and one of those heads is that of the chief editor of the journal (or of one of his associates) to which our manuscript had been submitted?

Possible, but unlikely. Let me ask you this: Did you have personal contacts with Professor Hruby at this time?

I did. Our contacts began in the following way. In January 1991, I got a letter from Dr. Kaubisch, Vice President of Selectide Corp. and he offered to visit us to discuss the possibilities of cooperation. He explained that Selectide was founded at the end of 1990 (well after our paper had been submitted) by Professor Hruby and others at

the University of Arizona. Dr. Kaubisch also mentioned in the letter that they saw the abstract of our presentation to the 10th International Symposium of Medicinal Chemistry (Budapest, 1988). Dr. Kaubisch came to Budapest in February 1991 and he explained that one of them found out that in the split-mix synthesis one peptide forms on each bead and they developed a screening method based on that. He invited me to visit Selectide to give a seminar and discuss the possibilities of cooperation. I accepted the invitation and my visit took place in early April 1991. I gave a seminar on April 2 at the Cancer Center of University of Arizona, Tucson, speaking about the split synthesis and the stepwise screening strategy I described in the 1982 document. Dr. Lam (first author), Professors Hruby and Salmon (director of the Arizona Cancer Center and Dr. Lam's boss) as well as other authors of their later papers and the earlier patent application were among the audience. After they asked me to sign a confidentiality document, with which I complied, they offered me a consultancy (which was never realized), and showed me their screening method. As a possibility of cooperation, however, they suggested to me to do analytical experiments with non-natural amino acids they used in peptide synthesis. I found this offer inappropriate and told them that our cooperation should be connected with our synthetic method. Finally we agreed to continue the discussions by mail but the cooperation was never realized because of later events.

When did you sense for the first time that something went wrong?

A few months after my visit to Tucson, one of my former students attended the 12th American Peptide Symposium in Boston in June 1991. She told me that Dr. LAM had an oral presentation reporting about the split-mix synthesis and their screening method [12] but made no reference to our work. Later on, one of my colleagues took part in the Symposium on the Innovation & Perspectives in Solid Phase Synthesis and Related Technologies in August 1991, where Richard Houghten presented our synthetic method as his own [13]. Yet later, I found in *Nature* the papers of LAM et al. [11] and HOUGHTEN et al. [14] that appeared in September 1991 and in which they described the split-mix synthesis as their own invention and without any reference to our papers.

How did you feel about this and what could you do in this situation?

I have to tell you that this situation has caused me much bitterness through the years. As my first reaction, I wrote a letter to both parties asking for a correction in *Nature* and citation of our papers.

What was their reaction?

Let me speak first about the Hruby group. Both Professor HRUBY and Dr. LAM wrote me an apologizing letter stating that in the first version of their *Nature* papers, there was a reference to our work but they had to shorten the manuscript and the

reference was deleted. They also promised to publish a correction in *Nature*. First of all I have to note that if you read the paper, you don't get the impression that anything was left out. They described the synthetic method as their own invention and in later papers they called it the Selectide process. After almost one year of delay, their correction, with an ambiguous content, appeared in *Nature* [15]. My name, however, was misprinted: Fukura instead of Furka. After repeated correspondence, a correction of the correction appeared in *Nature* in December 1992 [16].

I questioned the explanation of omitting the references to our papers by Hruby and Lam from the beginning. Later on, I found evidence that convinced me that omitting us from the list of references was intentional. They have published other papers and documents from which citation of our work is also missing. Let me mention only two examples.

(i) Around the time of publishing the *Nature* paper Professor Hruby and some of his co-workers wrote a chapter in a book [17] in which the split-mix synthesis is also mentioned without reference to our contribution.

(ii) Professor Sydney E. SALMON of the University of Arizona (one of the authors of the *Nature* paper [11]) had grant applications in cooperation with HRUBY and others based on the split-mix synthesis. The synthesis was indicated in their proposals as their own invention. One of the successful proposals to the National Institutes of Health had the title "Discovery of Peptide Anticancer Drugs," grant No. CA57723. This grant lasted from 1992 to 1995 and brought more than 3 million dollars to the applicants. A copy of the grant application was in my hands and I saw no reference in it to our work.

Let's turn now to Dr. HOUGHTEN. I also asked him to correct his *Nature* paper [14]. He promised to do that, called me by phone, and also offered cooperation and financial support. He sent me the copy of the correction letter supposedly mailed by him to *Nature*. He wrote in the letter 'As we were unaware of any of Dr. Furka's work we did not include it as a reference in our manuscript. We regret this omission . . .'. The correction, however, was never published. It deserves to look closer what Dr. HOUGHTEN states in his letter: 'we were unaware of any of Dr. FURKA's work.' I later found Dr. HOUGHTEN's patent application [8] in which he cited the abstract of our 1988 presentation in Prague [3]. The patent was filed on November 21, 1990, and the *Nature* paper was submitted on July 31, 1991. Let me ask you: Could anybody believe that what he knew in November 1990 was unknown to him eight months later?

Did you try to take other actions in addition to those outlined above?

Yes, but all of them were unsuccessful. I tried to publish myself a letter in *Nature*, but this was declined by Professor Maddox, who was the Editor at that time. I also contacted the Office of Research Integrity of the Department of Health & Human Services of the U.S. I had long correspondence with Dr. Alan Price of that office and sent him all the evidences. Finally, he concluded that in his opinion no plagiarism

occurred and advised me to turn to the University of Arizona. I also contacted the Publisher of the *International Journal of Peptide and Protein Research*, again without any success.

What was the effect of Drs. Hruby's and Houghten's actions on you?

In addition to the bitterness that lasted for years, for a long time only their papers were cited in the literature. Later on, and still nowadays, their work is often cited as independent from that of ours. I had difficulties for years in publishing papers and had no possibility to give oral presentations at peptide symposia. Many years later, a friend of mine who was chairman of a combinatorial session of a peptide symposium told me that he was instructed by the organizers not to give me possibility for questions or comments.

Despite all this, the situation has gradually changed. I remember that shortly after the appearance of Lam's and Houghten's *Nature* papers, I got a letter from Professor W. C. Still of Columbia University, asking me to clarify the priority question by sending him earlier publications. I sent him copies of the two 1988 abstracts [3, 4] and the 1982 document [1, 2]. I also made available copies of the 1982 document as a supplement to my posters presented at symposia. As a consequence, people have realized that the real birthplace of combinatorial chemistry was our laboratory in Budapest, and I have begun to be called the 'pioneer' or the 'father of combinatorial chemistry.' I was elected Honorary President of the European Society of Combinatorial Sciences when this organization held its first symposium in Budapest in 2001.

Some years ago, I already made an interview with you [18, 19] *and you also published a paper about the history of combinatorial chemistry* [20], *but you did not speak about these problems. Why?*

As mentioned before, my earlier efforts to clarify the priority problems were unsuccessful. What happened was a scandal in science and most people, including myself, do not like scandals. Also, it is not easy to speak about such events in science, particularly if you are the victim. My friends also advised me to be silent since those who speak out about such things, the whistle blowers, are often considered as bad guys. Lately, however, colleagues, whose opinion I respect, told me that it's time to bring out the truth to light.

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