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Combinatorial Chemistry*

In many areas of chemical synthesis, drug discovery, and materials research, combinatorial approaches are becoming more and more important. One of the most serious bottlenecks in this area is the chemical analysis and screening of the products. Was the correct product produced? Were all the possible products produced? What is their purity? Where in a combinatorial library are the 'hits'? If a relatively large number of lead compounds or hits have been identified, what is the most effective way for their thorough chemical analysis? The key goals of this symposium were to provide an overview over the current state-of-the-art analytical strategies used in combinatorial chemistry, and to show how obstacles can be overcome by new analytical tools and strategies. It was fascinating to experience how many areas of chemistry are influenced by combinatorial chemistry nowadays, and it was impressive to witness the efforts being made by software developers and chemists alike to overcome some remaining hurdles in this area.

Keywords: Analytical chemistry · Combinatorial chemistry · ILMAC

The first speaker was Professor *Mark Bradley* from Southampton University, UK. His talk was entitled **'Single Bead Screening and Analysis'**. Bradley started by describing different screening methods, both on and off single beads [1], for example zone diffusion type screening after cleaving a photolabile or pH-sensitive linker. He also showed analysis of cleaved compounds by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, including a ladder-type sequencing method based on methionine tags that are cleavable with CNBr. Bradley pointed out that for analytical assays that are not based on mass spectrometry, the compound loading per bead is generally not high enough. His group has tackled this problem by attaching amine-terminated dendrimeric groups onto the surface of the beads. This permits the loading to be increased to up to 700 nmol of compound per bead (for beads of 400 ... 500 μm in diameter). Bradley demonstrated in an impressive fashion that high quality HPLC and even NMR data can be obtained from single beads in this fashion. The screening of inhibitors of trypanothione reductase was shown as an example: K_i values from single beads were measurable in this way [2].

This was followed by a presentation given by Dr. *Günter Bovermann* from Novartis Pharma AG, Basel, Switzerland on **'Spectroscopy in the High-Throughput Age'**. With the introduction of combinatorial or multiple parallel synthesis techniques in medicinal chemistry, the number of samples to be analyzed has dramatically increased. In this situation, instrument manufacturers are proud to present equipment and technologies suitable for high-throughput acquisition. However, the automated measurement of thousands of spectra raises the question of how to handle evaluation or interpretation of huge amounts of data, a problem that has long been underestimated. In other words, Bovermann claimed, the bottleneck in structure elucidation has simply shifted from data acquisition to spectra interpretation. It is evident that the analytical task is not finished by acquiring data: a spectroscopy lab has to provide results, not data. The conventional – and yet by far predominant – way of spectroscopic data evaluation consists of manual interpretation of MS, NMR, and IR/Raman spectra, to a certain degree supported by expert systems or spectroscopic databases. Simple upscaling of the traditional process of manual interpretation would be completely impossible: if the interpretation of one spectrum takes five minutes on average, 96 spectra could be interpreted in one day, *i.e.* it would take ten highly trained full-time employees to interpret the required number of about 1000 spectra per day. Software tools for automated spectra interpretation do exist, but usually do not meet the reliability criteria required for unattended use. Thus, Bovermann's unit at Novartis reconsidered some of

The speakers and the session organizer. Front row (from left): Dr. S. Guan, Prof. P. Chen, Prof. M. Bradley; Second row (from left): Dr. R. Neudert, Dr. G. Bovermann, Prof. R. Zenobi

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the basic steps and principles of this process, which in turn allowed them to revise existing and to develop new programs for use in full automation. Bovermann showed examples of the USE software ('Unattended Spectra Evaluation'). He also showed how the 'Rack Viewer', a suite of programs developed in-house at Novartis, is used for visualization of data from whole microtiter plates. Finally, he pointed out that different spectroscopic methods (^{13}C NMR, ^{1}H NMR, IR, MS ...) are very different with respect to their sensitivity, sample handling requirements, data analysis, and automatic data interpretation. This is reflected in very different potential for their use as completely automated methods, and therefore, for their application to problems in combinatorial chemistry.

Dr. *Shenheng Guan* from Symyx Technologies, Inc. (Santa Clara, CA, USA) gave the next talk, entitled '**High-Throughput Screening of Combinatorial Heterogeneous Catalyst Libraries**'. Symyx Technologies is active in producing and analyzing novel materials, using strategies of combinatorial chemistry, for applications in a variety of fields, *e.g.* luminescent materials, superconducting films, or heterogeneous catalysts. The application of combinatorial chemistry methods to the discovery of new materials for these purposes requires a means of rapidly testing their performance. In catalysis, this is typically accomplished through a series of screens (termed primary, secondary, and tertiary) that are designed to identify and test the most promising compositions and reaction conditions of a large group of potential catalysts. In the exploratory primary screen, only a few important variables, such as selectivity and activity, are measured. Though limited in terms of reaction parameters, the primary screen provides useful results with maximum throughput. The secondary screen is used to validate and test more completely the candidates identified in the primary screen and to optimize certain materials-related variables, such as support and metal loading, and process conditions, such as temperature and residence time. Consequently, the secondary screen should more closely approximate industrial reactor conditions but still maintain a reasonable throughput.
Guan's talk addressed the design and fabrication of primary and secondary screening tools capable of measuring the activity, selectivity, and space time yield from catalysts contained in tens to hundreds of parallel reactors. A very interesting apparatus that Symyx uses in this context is a 'scanning mass spectrometer', a device whose sampling orifice can be accurately positioned over an area containing a library of potential catalysts materials on a wafer. The throughput of the sampling orifice, as well as the height above the surface of the catalyst were found to be of particular importance for this device to function effectively. The results demonstrate that these tools can be used to rapidly accelerate discovery, for example for ethylene hydrogenation catalysts. An important step is to use large libraries. Guan showed a microfabricated reactor for massively parallel screening of catalyst libraries.

'**Combinatorial Chemistry – from Data to Information**' was the topic of Dr. *Reinhard Neudert* from Chemical Concepts/ Wiley, Weinheim, Germany. Combinatorial chemistry was presented as a new challenge for spectroscopic laboratories, because of the large number of compounds, available only in microgram amounts, that have to be characterized by analytical techniques. Several aspects of this task were discussed in view of the present state and future developments in this area.
An important step concerns the verification of the results. The classical way is to delegate this task to a spectroscopist who decides if a structure proposal is confirmed by a given spectrum. In case of several hundreds or even thousands of verifications per day this is not feasible. Computer software for automatic verification has to be applied. Verification modules for ^{13}C NMR, ^{1}H NMR, IR, and MS spectra were discussed. In the first three cases the verification is based on spectrum prediction techniques. MS can be verified by using empirical rules, isotope patterns, fragmentation, and fragment compositions.
Finally the verified data have to be stored in a central data archive together with the verification results. The chemist's interest is to rapidly obtain information whether a synthesis resulted in the expected products. Neudert described a very interesting and convenient Web-Client system (written in JAVA and therefore platform-independent) for easy display of the verification results on a personal computer. The original spectroscopic data can be retrieved from a central data server, either located inside a company (for example, if one has to deal with proprietary compounds) or alternatively outside the 'firewall'. This Web-Client system will upload spectroscopic data from the user's personal computer, using the JCAMP format or native binary formats. It will then be compared to database spectra. Wiley, for example, the parent company of Chemical Concepts, offers spectral databases of Chemical Concepts containing over 660 000 spectra in eighteen different database sets as part of their InterScience program. Chemical structures as well as many types of spectra are available in these databases, including NMR, MS, IR, Raman, NIR, UV, and even 2D-NMR (in the next release).

The concluding presentation was given by Prof. *Peter Chen* from the ETH Zürich, Switzerland. Chen has also formed a company recently, Thales Technologies AG, whose goal is high-throughput screening and synthesis of polymerization catalysts. His talk was entitled '**High-Throughput Screening of Catalysts by Electrospray Ionization Tandem Mass Spectrometry**'.
Chen showed the application of a new mass spectrometric method to the rapid screening of both pooled and parallel libraries of polymerization catalysts [3]. It is based on being able to use electrospray ionization mass spectrometry to observe the growing polymer still bound to the polymerization catalyst. In the tandem MS variant, these complexes are collided with a noble gas inside a collision chamber and the bare catalyst is observed. Chen showed how known mass spectrometric tricks, for example parent ion scans after passing the ions through a high mass filter, can be exploited to find the most successful catalyst (in this case defined as the one producing the highest molecular weight polymers) can be identified in a mixture/library. If the mechanism and the kinetics controlling the polymerization are understood, it is even possible to predict properties of a polymer (such as the average molecular weight or the molecular weight distribution) for the actual conditions of an industrial polymerization process. The method was demonstrated with single-site Ziegler-Natta and ring-opening metathesis polymerization catalysts. Screening can be done in solution with mg quantities of catalysts, *in situ* activation, and requires only a few minutes.

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