UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

INTERNATIONAL CENTRE FOR SCIENCE AND HIGH TECHNOLOGY, TRIESTE, ITALY

AMBASCIATA D'ITALIA BUDAPEST. HUNGARY

EÖTVÖS UNIVERSITY, BUDAPEST

ICS-UNIDO Workshop on

Trends and Applications of Combinatorial Chemistry and Combinatorial Technologies

Budapest, Hungary 15–18 October, 2001

Co-sponsors:

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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

INTERNATIONAL CENTRE FOR SCIENCE AND HIGH TECHNOLOGY

ICS Workshop on

"TRENDS AND APPLICATIONS OF COMBINATORIAL CHEMISTRY AND COMBINATORIAL TECHNOLOGIES"

Budapest, Hungary 15–18 October, 2001

Provide the participants from the region with updated knowledge on modern technologies **and state-of the-art overviews** on the **recent developments** in the field of combinatorial chemistry and combinatorial technology. Problems related to combinatorial science running on as result of industrial and scientific development in the countries of Central-Eastern Europe will be discussed. The workshop will be based on theoretical lectures, practical demonstrations, case studies, interactive small-group seminars, and interactive problem-solving exercises. Stimulate international research and technology transfer and enhance international co-operation through possible joint or follow-up projects and feasibility studies by identifying regional R&D&I Centers in the region through contacts established with the participants of the workshop, thus giving ICS the possibility of identifying qualified and academic centers for future joint ventures.

Participation is open to scientists, researchers, postgraduate students, government administrators, industrialists and managers involved in the field of combinatorial science or willing to introduce the adequate modern combinatorial technologies in their countries. Preference should be given to participants who actively participate in their countries research programmes using tools of combinatorial chemistry and who are involved in their implementation.

The workshop is sponsored by ICS-UNIDO. **There is no registration fee.** Travel and living expenses will be free for a limited number of participants selected by ICS-UNIDO. Self-financed participation is encouraged.

Scientific Committee of the Workshop: Prof. Gábor Dibó (Eötvös University, Hungary), Prof. Stanislav Miertus (ICS-UNIDO), Dr. Giorgio Fassina (Italy), Dr. Pierfausto Seneci (Germany).

The closing date for requesting admission is 15 September 2001. More information: Prof. Gábor Dibó (Ph: +36-1-372-2771; Fax: +36-1-372-2620; E-mail: dibo@szerves.chem.elte.hu**)**

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

INTERNATIONAL CENTRE FOR SCIENCE AND HIGH TECHNOLOGY

supported by the Italian Embassy in Budapest

AMBASCIATA D'ITALIA BUDAPEST

SUNDAY

October 14, 2001

12:00 – 18:00 REGISTRATION

Eötvös University, Faculty of Science
Chemistry Building, Gate 1/A
Pázmány Péter sétány 1/A
Budapest, H-1117

18:00 DEPARTURE FOR HOTEL AURA (Symposium venue)

Methodology and Information Centre for In-service Teacher Traning of the Ministry of Education
Pilisborosjenô Fô út 1.
Pilisborosjenô, H-2097

19:00 HOTEL REGISTRATION

19:15 Dinner

MONDAY

October 15, 2001

7:30 – 8:30 Breakfast

8:30 – 9:00 **Stanislav MIERTUS** *(ICS-UNIDO, Trieste, Italy)*
ICS-UNIDO Programmes – An Introduction

9:00 – 10:45 **Giorgio FASSINA** *(Xeptagen SpA, Naples, Italy)*
Combinatorial Technologies – An Overview

10:45 – 11:00 Coffee Break

11:00 – 12:45 **Alexey, ELISEEV** *(State University of New York, Buffalo, NY, USA)*
Dynamic Combinatorial Libraries

12:45 – 13:15 Discussion

13:15 – 14:15 Lunch

14:15 – 16:15 **Claude MIRODATOS** *(CNRS, Villeurbanne, France)*
Combinatorial Optimization of Heterogenous Catalysis

16:15 – 16:30 Coffee Break

17:00 – **WELCOME RECEPTION**

In the Aula of the Methodology and Information Centre for In-service Teacher Training of the Ministry of Education,
Pilisborosjenô, Fô út 1.

TUESDAY

October 16, 2001

7:30 – 8:30 Breakfast

8:30 – 9:30 **Wolfgang BENDER** *(Bayer AG, Wuppertal, Germany)*
The Bayer Synthon Concept

9:30 – 10:30 **Ferenc HUDECZ** *(Hungarian Academy of Sciences, Budapest, Hungary)*
Application of MS for Library Characterization

10:30 – 10:45 Coffee Break

10:45 – 11:45 **Giorgio FASSINA** *(Xeptagen SpA, Naples, Italy)*
Biological Methods for Library Characterization and Screening

11:45 – 12:45 **István T. HORVÁTH** *(Eötvös University, Budapest, Hungary)*
Application of Fluorous Biphase Chemistry in Combinatorial Technology

12:45 – 13:15 Discussion

13:15 – 14:15 Lunch

14:15 – 15:15 **István GREINER** *(Richter Gedeon, Budapest, Hungary)*
Robotics & Lab Automation

15:15 – 16:15 **László KOVÁCS** *(InFarmatik, Budapest, Hungary)*
Combinatorial Process Research & Development

16:15 – 16:30 Coffee Break

16:30 – 18:30 **Wolfram ALTENHOFEN** *(Chemical Computing Group, Lörrach, Germany)*
QSAR Modelling to Library Design Strategies

18:30 – 19:30 Dinner

19:30 – **Free Time**

WEDNESDAY

October 17, 2001

7:30 – 8:30 Breakfast

8:30-9:30 **Menotti RUVO** *(Xeptagen SpA, Naples, Italy)*
Combinatorial Chemistry in Biotechnology - A Case Study

9:30-10:30 **Béla NOSZÁL** *(Semmelweis University, Budapest, Hungary)*
Combinatorial Phenomena in Biological Systems

10:30 – 10:45 Coffee Break

10:45-12:45 **Pierfausto SENECI** *(NAD AG, München, Germany)*

Molecular Diversity in Drug Discovery: A Critical Assessment

12:45 – 13:15 Discussion

13:15 – 14:15 Lunch

14:15 – 16:15 **Aubrey MENDONCA** *(Polymer Laboratories, Amherst, MA, USA)*
Solid Phase Synthesis – An Overview

16:15 – 16:30 Coffee Break

16:30 – 17:30 **Aubrey MENDONCA** *(Polymer Laboratories, Amherst, MA, USA)*
Solid Phase Synthesis – Recent Developments in Resin Technology

17:30 – 18:30 **Péter ARÁNYI** (Chinoin-Sanoffi, Budapest, Hungary)
Role of Combinatorial Chemistry in Original Drug Discovery

18:30 – 19:30 Dinner

19:30 – **Free Time**

THURSDAY

October 18, 2001

7:30 – 8:30 Breakfast

8:30 – 10:15 **Peter van den BRINK** *(Avantium Technologies BV, Amsterdam, The Netherlands)*
High Throughput Technologies: An Exciting New Development in Process Chemistry Research and Development

10:15 – 10:30 Coffee Break

10:30 – 12:30 **György KÉRI** *(Semmelweis University, Budapest, Hungary)*
Rational Drug Design and Signal Transduction Therapy
11:30 – 12:30
12:30 – 13:30 **György DORMÁN** *(ComGenex, Budapest, Hungary)*
Good Quality Libraries (Predicted and Measured Parameters)

13:30 – 14:15 Lunch

14:15 – 15:45 **COUNTRY REPORT**

15:45 – 16:00 Coffee Break

16:00 – 17:30 **FOLLOW-UP SESSION**

17:30 – 18:30 **Árpád FURKA** *(Eötvös University, Budapest, Hungary)*
Twenty Years in Combinatorial Chemistry

18:30 – **BANQUETTE**

Abstracts

in aplhabetical order

QSAR MODELING AND LIBRARY DESIGN STRATEGIES

Dr. Wolfram Altenhofen
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wolfram@chemcomp.de

The session will be devided into an introduction to basic concepts of QSAR Modeling and Library Design and a hands-on tutorial which will allow participants to experience the basic steps from deriving a QSAR model to designing a focused library themselves.
In the theory section, a general overview on

- representation of chemical structures in the context of computer applications,
- deriving physico-chemical properties
- the theory of ligand-protein interactions
- building QSAR models
- strategies for library design
- will be presented.

During the tutorial, a methodology is presented that guides through the drug design cycle starting from the analysis of experimental HTS data, constructing a QSAR model and using the model to design a virtual focused combinatorial library for cyclic GMP Phospho-diesterase V inhibitors in an almost fully automated way.

The analysis of the experimental dataset is based on 2.5D descriptors. These descriptors are fast and easy to calculate since they rely on 2D information and still reflect 90 % of the information inherent in 3D structures. They were specifically designed to provide a tool for a rapid though stable initial approach to large datasets of unknown SAR. The descriptor values correspond to binned van-der-Waals surface areas. The binning procedure was based on logP, MR and partial charge (PEOE), supposed to be fundamental physico-chemical properties that cover most of the relevant property space in an intuitive and interpretable manner.

The QSAR model applies a non-linear probabilistic binary method rather than a linear regression based technique. The focused library design uses virtual enumeration with a binary QSAR model as product-based scoring agent for reagent selection.

The dataset consists of about 400 known cGMP Phosphodiesterase V inhibitors with activity data selected from the literature and a total of 1800 molecules. The initial QSAR model is about 20 times more potent in selecting active compounds over random picking. The building blocks (2 x 10 x 12 x 27 = 6500 potential products) used in the combinatorial design of a focused quinazoline library (1 x 3 x 3 x 5 = 45 products) reflect chemical intuition and input from the literature. Using the binary QSAR model as focusing agent the percentage of predicted active compounds increases from 5 % in the unfocused library to 75 % in the focused library. The resulting focused library preserves the essential SAR known from the literature.

Role of Combinatorial Chemistry in Original Drug Discovery

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Combinatorial synthetic methods became a routine in drug discovery during the nineties. Use of combinatorial libraries find two well discernible applications. In order to identify random hits, a diverse combinatorial library can be added to in-house existing compounds and tested in first screen assays. Later in the discovery process a focussed library is more useful to optimize the structure in order to get a lead. Several different technical solutions exist today. The most straightforward approach apparently is parallel synthesis of individual compounds. An aspect that should be considered while designing the basic scaffold (and set of substituents) is drug-likeness of the resulting compounds. Known toxicophores, mutagenic cores, alkylating, acylating or other highly reactive side chains should be avoided. Molecular weight of the compounds should remain below or in the vicinity of 500. Many published libraries are built around core structures of known drugs on the market or in development. Structures that are not stable in the biological milieu, or otherwise have poor bioavailability, such as peptides or alkyl esters are defavorized even if their chemistry is easy to master.

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Dynamic Combinatorial Chemistry

Presented by Alexey Eliseev

The major effort of today's combinatorial chemistry is focused on the synthesis and screening of libraries of individual compounds. The alternative approach, use of mixtures (pools) of compounds, is significantly less labor and resource consuming, but requires elaborate analytical tools to identify effective components in complex mixtures.

This lecture will consider dynamic combinatorial chemistry (DCC), an approach to molecular diversity generation and screening that involves reorganization of pools of compounds, existing in a dynamic equilibrium, *via* their interactions with the target compound. Such reorganization results in the formation of amplified amounts of those components that form the strongest complexes with the target and thereby simplifies their isolation and identification. DCC offers a potentially new approach to drug discovery that combines library synthesis and screening in a single step and allows one to rapidly explore and customize pharmaceutical diversity space for a given target.

The following subjects will be considered in the presentation.

1) DCC as a general approach to synthesis and screening of combinatorial libraries: advantages and limitations as compared to parallel techniques.

   A. Case studies of early examples of dynamic libraries. Bioactive peptides, cation receptors, inhibitors of carbonic anhydrase.
   B. Mechanisms and quantitative assessment of amplification effect in dynamic libraries. Thermodynamic vs. kinetic effects.
   C. Basic reactions used in DCC. Examples of imine exchange, transesterification, coordination chemistry, alkene metathesis.

2) DCC as emerging tool of drug discovery. Case study of neuraminidase inhibitors formed from*in vitro* virtual libraries.

3) Other applications of dynamic libraries.
   A. Nucleic acid recognition.
   B. Ion separation.

4) Methodological developments in DCC:
   A. Dynamic deconvolution.
   B. Multi-level dynamic libraries.
   C. Analytical techniques: case study of regiochemical tagging.

Suggested Literature

1. A. Ganesan, Angew. Chem. Int. Ed. Engl. 37, 2828-2831 (1998).

2. J. M. Lehn, Chem. Eur. J. 5, 2455-2463 (1999).

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Combinatorial Technologies – An Overview

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The time and cost needed for the development of new drugs have increased steadily during the past three decades. Estimated costs for introducing a new drug in the market now reach around 200-300 millions USD, and this process takes around 10-12 years after discovery. This increase in time and cost is due mainly to the extensive clinical studies of new chemical entities required by competent regulatory agencies, such as the FDA, and to a lesser extend to the increased costs associated to research. The time and cost required for clinical and preclinical evaluation of new drugs is not likely to decrease in the near future, and as a consequence, a key issue for pharmaceutical companies to stay in the market has been to increase the number of new drugs in the development pipeline. Drug discovery in the past has been based traditionally on the random screening of collection of chemically synthesized compounds or extracts derived from natural sources, such as microorganisms, bacteria, fungi, plants, of terrestrial or marine origin or by modifications of chemicals with known physiological activities. This approach has resulted in many important drugs, however the ratio of novel to previously discovered compounds has diminished with time. In addition, this process is very time consuming and expensive. A limiting factor was linked to the restricted number of molecules available or extract samples to be screened, since the success rate in obtaining useful lead candidates depends directly from the number of samples tested. Chemical synthesis of new chemical entities often is a very laborious task, and additional time is required for purification and chemical characterization. The average cost of creating a new molecular entity in a pharmaceutical company is around 7500 USD/compound. Generation of natural extracts, while very often providing interesting new molecular structures endowed with biological properties, leads to mixtures of different compounds at different concentrations, thus making activity comparisons very difficult. In addition, once activity is found on a specific assay, the extract needs to be fractionated in order to identify the active component. Quite often, the chemical synthesis of natural compounds is extremely difficult, thus making the lead development in to a new drug a very complex task. While the pharmaceutical industry was demanding more rapid and cost effective approaches to lead discovery, the advent of new methodologies in molecular biology, biochemistry, and genetic, leading to the identification and production of an ever increasing number enzymes, proteins, receptors, involved in biological processes of pharmacological relevance, and good candidates for the development of screening assay, complicated even more this scenario. The introduction of combinatorial technologies provided an unlimited source of new compounds, capable to satisfy all these needs. This approach was so appealing and full of promises that many small companies started to flourish financed by capitals raised from private investors.

Combinatorial approaches were originally based on the premise that the probability of finding a molecule in a random screening process is proportional to the number of molecules subjected to the screening process. In its earliest expression, the primary objective of combinatorial chemistry focused on the simultaneous generation of large numbers of molecules and on the simultaneous screening of their activity. Following this approach, the

success rate to identify new leads is greatly enhanced, while the time required is considerably reduced.

The development of new processes for the generation of collection of structurally related compounds (libraries) with the introduction of combinatorial approaches has revitalized random screening as a paradigm for drug discovery and has raised enormous excitement about the possibility of finding new and valuable drugs in short times and at reasonable costs. However the advent of this new field in drug discovery did not obscure the importance of "classical" medicinal chemistry approaches, such as computer-aided rational drug design and QSAR for example, but catalyzed instead their evolution to complement and integrate with combinatorial technologies.

Combinatorial Process Research & Development

László Kovács
InFarmatik
Hungary

Abstract

Introduction:
The accelerated drug discovery and increasing outsourcing have increased the importance of the Process Research & Development (P R&D) in the pharmaceutical industry. Beside the obvious direct benefit of reducing manufacturing cost of the drugs, other useful applications were find for P R&D. Since combichem provide methodology and tools: labware, automation, software, and complete instrumentation, the automated P R&D brought a lot of results quickly.

Discussion:
The lecture deals only with real combinatorial part of automated PR&D: process scouting and process optimization. In these stages vary large parameter (factorial) field should be mapped.
In order to be able to deal with this large factorial field one should combine the following feautres:

5) Parallel synthesis reactors
6) Liquid handlers
7) Analysis
8) Control software
9) Design of experiments

Since temperature is a key factor in chemical reactions and properties beside the traditional isotherm block reactors, the manufacturers have developed machines with thermal zones or individual heating and cooling.
Integrated systems control the whole procedure from preaparation of reactions till collecting the data from the analyisis (mostly HPLC) detecors(s).
The control software is a key issue in these systems, since rational handling of limited resources might be a key issue in the success.
Design of experiments can substantially reduce the number of experiments, needed to find the optimum of a process.
The examples are collected to cover the whole range of the affected pharma and agro industry, from the discovery till the manufacturing of active substances. Different methods for optimum search are demonstarted.

ICS-UNIDO Workshop
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Combinatorial approaches for speeding up heterogeneous catalyst discovery and optimisation: strategies and perspectives for academic research.

Claude Mirodatos

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Over the past five years, combinatorial chemistry applied to heterogeneous catalysis has been dealt with in more and more articles, reviews and patents . This methodology remains very controversial, however. Today, within universities as well as within public and private research centres, attitudes toward combinatorial methods run the gamut from fascination to scepticism (or even outright rejection). The debate usually originates from a misunderstanding of the strategies at hand. As such, "combinatorial catalysis" is too often mistaken for a random, undisciplined mixing of various chemicals. On the contrary, the combinatorial approach embodies conventional catalysis, micro mechanics, robotics, analytical methodology and information technology.

Industry essentially seeks to use the combinatorial approach in order to accelerate the discovery of new materials and reduce time-to-market, and this is generally well accepted. The role of academia, however, remains a matter of debate. Some of the most frequently asked questions are:

- Is combinatorial catalysis an accelerated conventional process for catalyst preparation or a new methodology?
- Does academic combinatorial research aim only at discovering entirely new materials?
- Are creativity and fundamental knowledge still required of scientists?

This presentation aims to clarify the debate.

The application of combinatorial chemistry to heterogeneous catalysis is analysed in terms of current strategies and perspectives on the industrial and academic levels. Potential methodologies for academic research laboratories are proposed with emphasis on both theoretical and practical considerations.

As a case study, the European consortium "COMBICAT" "Catalyst Design and Optimisation by Fast Combinatorial Procedures" is presented focusing on the chosen strategy [1].

"COMBICAT" started on 01/01/00 is dedicated to the "Competitive and Sustainable Growth" EU programme. It mainly deals with the development of innovative combinatorial methods of fast preparation and high-speed testing of solid materials to be used as heterogeneous catalysts to reduce R&D time and costs. The new methods to be developed will

be validated using a widespread of catalytic reaction categories of importance for European chemical industry.

In that consortium, 10 research partners (3 large companies, 2 SME, 4 research institutions, 1 university) from 6 European countries are grouped to fulfil the work program. The partners cover all point of views within the project: Research institutions with widespread basic knowledge on catalyst development, experienced SME´ sas specialists for development of chemical research software and high-tech robotics hardware and large catalyst production companies as well as catalyst end users (engineering entities) of the European chemical industry.

Various aspects of the running research will be presented:

- analysis of the combinatorial approach to heterogeneous catalysis,
- strategies and technologies for secondary screening,
- preparation and testing of catalyst libraries : development of hard and software tools adapted to case studies
- strategies for a combinatorial approach of kinetic modelling, applied to transient operations.

All these key steps in the combinatorial approach for heterogeneous catalysis may be summarised in the following scheme presented in Fig. 1.

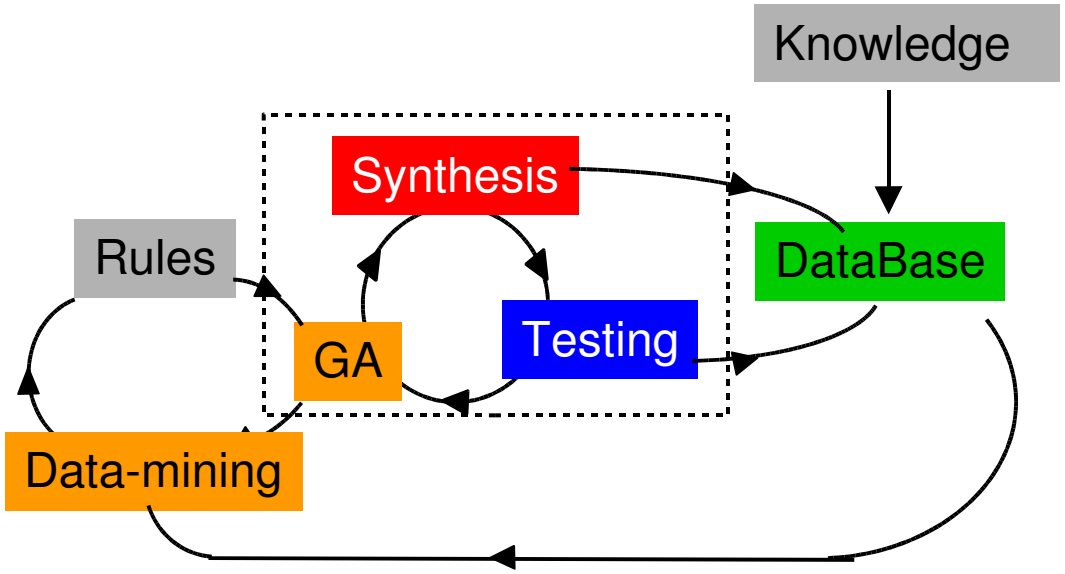

Fig 1: Improved strategy for catalyst optimization which combines an iterative methodology with data mining techniques. The dashed square shows the conventional approach.

As a general conclusion, the importance of robotics with respect to scientific creativity is likely overestimated in the HT approach. Most breakthroughs speeding up the discovery of new materials will not likely come from faster or highly parallel techniques, but probably from smart ideas allowing synthesis, screening and further optimisation via data mining.

This last observation drives home the point that research in combinatorial catalysis is still at an early stage, on the threshold of many possible applications. In the future, when combinatorial catalysis has matured, the scientist's preoccupation will shift toward setting up appropriate screenings as well as tuning and selecting appropriate, powerfully data handling

software. In the meantime, enormous initial efforts and time will be required to develop both technological tools and efficient strategies.

Combinatorial catalysis is not a new field in science, but an interdisciplinary topic involving many different research communities. We believe that its success relies on combining scientist creativity and advanced technology, which should lead both to new breakthroughs and to a broadened understanding of catalysis [2,3].

Acknowledgements: D. Farrusseng, L. Baumes, I. Vauthey, C. Hayaud, P. Denton are fully acknowledged for their efficient participation to that work, and the EU "Combicat" programme for supporting part of the quoted work.

References :

[1] website of COMBICAT programme : www.ec-combicat.org

[2] Combinatorial approaches to heterogeneous catalysis: strategies and perspectives for academic research, A. Holzwarth, P. Denton, H. Zanthoff and C. Mirodatos, Catalysis Today 2441 (2001) 1-10.

[3] The combinatorial approach for heterogeneous catalysis: a challenge for academic research. D. Farrusseng, L. Baumes, I. Vauthey, C. Hayaud, P.Denton, C. Mirodatos, To appear in the proceedings of the NATO-ASI Conference, July 16-27/2001, Vilamoura, Portugal

COMBINATORIAL PHENOMENA IN BIOLOGICAL SYSTEMS

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Combinatorial chemistry (C.c.) is a recent branch of sciences, with several applications in drug research.
C.c. produces a wide variety of compounds, in order to provide the target moiety of the drug receptor with a large selection of possibly binding countermolecules.

The number of compounds formed can be expressed in terms of combinatorics, such as the number of combinations, variations, permutations, and numerous exponential formulas.
For example, if pentapeptide libraries are produced using 7 amino acids, the number of constitutionally distinct peptides is 7^5 (the number of combinations regardless the sequence). The possible, non-repeating sequences within a given set of five amino acids are 5! = 120, the number of permutations, which allows for 2520 pentapeptides of 5 different amino acids each. If repeating sequences are also permitted, the total number of pentapeptides with 7 building blocks is 7^5 = 16807. Such cornucopia of compounds represents a substantial chance of receptor binding.
Several analogous combinatorial phenomena occur in biological systems.
Two of such combinatorial events are the protonation and conformation changes of biomolecules, in which a wide variety of distinct species are formed in a spontaneous manner. Prime examples are the neutrotransmitters that constitute an extremely important group of versatile, multiconform biomolecules.

These compounds are typically of low molecular mass and relatively few atoms, but they usually bear several biological functions, due to their structural and coulombic chageability, and the concomitant set of distinct forms that can be counted by operations of combinatorics.

For example, glutamic acid, one of the 20 ˝classical˝ amino acids and a ubiquitous neurotransmitter on excitatory amino acid receptors, carries at least 6 biological functions, which can be assigned to its $F = 2^n . 3^m$ different solution forms, where n is the number of basic sites, and m is the number of rotational axes. For glutamic acid, n = 3, m = 2, and F = 72.

All the 72 forms of glutamic acid coexist in solution, providing the various receptors with a multitude of binding choices, being each of them is a particular microform of glutamic acid. The various microforms have different physico-chemical properties, with individual capabilities not only in receptor binding, but also in enzyme-catalysis, metabolism and membrane penetration. The significance, methods and results of combinatorial phenomena in biological systems will be further exemplified on N-acetylcysteine, the most widely used mucolytic agent[1], and amphetamine, a psychostimulant drug[2].

[1]Noszál, B., Visky, D., Kraszni, M.: *J. Med. Chem.* 2000, 43, 2176-2182
[2]Noszál, B., Kraszni, M.*: J. Phys. Chem. B*. 2001, in press

iological Methods for Library Characterization and Screening

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Biological methods for library preparation are mainly limited to peptide or oligonucleotide libraries. For peptide libraries, methods are based on the construction of a pool of clones each one expressing a different peptide on its surface. The peptides are fused to proteins normally expressed on the surface of the microorganism used. Phage display libraries are the most commonly used. Screening is accomplished by incubation of the target molecule, adsorbed to a solid support, with the phage population. Active phages will bind the target even after extensive washing steps. Target-bound phages are isolated and propagated by infection of E. coli and subjected to an additional round of adsorption to the immobilized target. This procedure increases both the number of active phages and the stringency of selection, since harsher condition may be employed in the washing steps to reduce the number of non-specifically bound phages. As for the case of synthetic libraries, iterative cycles of adsorption, washing, elution and propagation in E. coli are performed to enrich the phage population in the active or in few active sequences. Active phages may then be subjected to DNA sequencing in order to decode the active peptide sequence. In a very similar way, also oligonucleotide libraries can be screened for immobilized targets using the polymerase chain reaction (PCR) methodology to expand the number of active sequences after each selection cycles.

The construction of biological display libraries requires the introduction into a micro-organism of the genetic information necessary for the peptide synthesis . For the construction of a random peptide display library it is necessary to synthesize pools of DNA fragments that are then inserted into specific vectors. The DNA fragments are chemically synthesized as a mixture of single-stranded degenerated oligonucleotides containing constant regions and one or more degenerated stretches of DNA. DNA consists of sequences of 4 different nucleotides and each trinucleotide codes for a corresponding amino acid. Because of the codon degeneracy, most of the amino acids are coded by more than one triplet. Since in fully degenerated oligonucleotides there is the possibility to introduce stop codons that will interrupt protein synthesis, the oligonucleotides are synthesized using different mixtures of nucleotides especially in the third position of each triplet. The DNA fragments to be cloned must be in a double-stranded form, at least at the end of each fragment. This is normally done by annealing short oligonucleotides to a complementary constant region inserted during the synthesis and by enzymatically completing the complementary DNA strand. After compatible ends are prepared by restriction enzyme digestion, the fragments are ligated into an appropriate vector and then introduced into the microorganism.

The ligand selection process is called Biopanning. The target molecule must be bound to a solid support, usually a microtiter plate or a small Petri dish. Less common alternative supports are magnetic particles, column with solid matrices, cells, mammalian organs. In a typical experiment, the number of phages that are incubated with the target corresponds to about 100 to 1000 times the complexity of the library. After the unbound clones are washed away, the bound ones are eluted by different methods, like low pH, high concentration of free

target, direct infection of bacteria cells. The eluted phages are grown, purified and submitted to a new cycle of selection. Usually 3 to 4 rounds of selection are sufficient, and the entire process can be completed in about a week. At the end, several clones are isolated and their DNA extracted and sequenced. The DNA portions coding for the peptides are translated into amino acids and the sequences compared. If a consensus sequence can be identified, the screening may have been successful. One or more peptides are chosen and chemically synthesized in order to verify their binding affinity, outside of the microorganism system.
Compared to chemical libraries, biological display libraries have several advantages and disadvantages. Some of the major advantages are the possibility to use a library for many different selection processes (even 100s), the easy propagation of the library and of the selected clones. The possibility to build larger size libraries is another advantage together with simple selection and sequencing procedures. On the contrary, a disadvantage is the fusion of peptides to a microorganism protein, and, therefore, the binding site can be extended to the fusion protein or the fusion protein may influence the peptide conformation.

Suggested readings

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Combinatorial Chemistry in Biotechnology - A Case study

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Monoclonal antibodies are becoming an important class of therapeutic agents useful for the treatment of a vast array of diseases. Many monoclonals are waiting for FDA approval, and they represent almost 30 % of biotechnology derived drugs under development. Production of MAb's by hybridoma technology or transgenic animals can be easily scaled up, but still immunoglobulins purification from crude feedstocks poses several problems. Main difficulties are due to the low antibody concentration in cell culture supernatants or milk of transgenic animals and the high amounts of contaminating proteins. Purification by affinity chromatography of monoclonal antibodies for therapy is based on the use of protein A or protein G immobilized on appropriate supports [**1**], as a first step to capture and concentrate the immunoglobulin from diluted feedstocks. These two proteins, which bind to the constant portion of the immunoglobulins, and so can be used to purify the majority of antibodies, are obtained from microorganisms or genetically modified bacteria, trough complex and expensive procedures, requiring in addition time consuming analytical controls to check for the presence of contaminants such as viruses, pirogens, or DNA fragments, which may affect the safety of the purified MAb for clinical purposes. Given the importance of the application of MAb's for therapy, and given the role of the purification process in assuring the quality, consistency and safety of the products, it is clear that the availability of synthetic ligands able to mimic protein A or G in the purification of antibodies is of remarkable industrial importance, since may lead to less expensive production costs and reduced risks of contamination. A synthetic ligand [Protein A Mimetic, PAM], able to mimic protein A in the recognition of the immunoglobulin Fc portion, has been previously identified in our laboratory through the synthesis and screening of multimeric combinatorial peptide libraries [**2**]. Its applicability in affinity chromatography for the downstream processing of antibodies has been fully characterized, examining the specificity and selectivity for polyclonal and monoclonal IgG derived from different sources. Ligand specificity is broader than protein A, since IgG derived from human, cow, horse, pig, mouse, rat, rabbit, goat, and sheep sera [**3**], as well as IgY derived from egg yolk [**4**], are efficiently purified on PAM-affinity columns. Adsorbed antibodies are conveniently eluted by a buffer change to 0.1 M acetic acid or 0.1 M sodium bicarbonate pH 9 with full retention of immunological properties. Monoclonal antibodies deriving from cell culture supernatants or ascitic fluids are also conveniently purified on PAM-affinity columns, even from very diluted samples. The ligand is useful not only for IgG and IgY purification from different sources, but also for IgM [**5**], IgA [**6**], and IgE [**7**] isolation from sera or crude cell supernatants.

Affinity constant for PAM:IgG interaction is 0.3 M, as determined by plasmon resonance experiments. Antibody purity after affinity purification is close to 95 %, as determined by densitometric scanning of SDS-PAGE gels of purified fractions, and maximal column capacity reachs 30 mg Ig/ml support under optimized conditions. Validation of antibody affinity purification processes for therapeutic use, a very complex, laborious, and costly procedure, is going to be simplified by the use of PAM, which could reduce considerably the presence of

biological contaminants in the purified preparation, a very recurrent problem when using recombinant or extractive biomolecules as affinity ligands. In vivo toxicity studies in mice indicate a ligand oral toxicity >2000 mg/kg, while intravenous toxicity is close to 150 mg/kg [**8**]. Additional studies have suggested that PAM, given its ability to interfere with Protein A/immunoglobulin interaction, may find applications also as a novel therapeutic agent.

Protein A is the bacterial receptor for IgG, and this protein binds to IgG in a site partially overlapping with that of immunoglobulin receptors (Fc R). In further studies, a PAM derivative stable to proteolysis, prepared by replacing the natural amino acids with the corresponding D analogues, has shown to inhibit IgG/ Fc R in vitro in a dose dependent manner. Inhibition of Fc R is important in a wide range of diseases, such as Systemic Lupus Erythematosus (SLE). Administration of this derivative to MRL/*lpr* mice, the animal model to study SLE, has resulted in a remarkable enhancement of the survival rate (80 %) compared to placebo treated animals (10 %) and the significant reduction of proteinuria, the typical clinical sign associated to SLE. Kidney histological examination of treated animals has confirmed the preservation of tissue integrity and a remarkable reduction of immune-complexes deposition [**8**]. These results have confirmed the role of Fc receptors in SLE pathogenesis opening new perspectives for the development of new drugs for treating autoimmune disorders.

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Molecular diversity in Drug Discovery: a critical assessment

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This Lecture will at first examine the phases of modern drug discovery and see where diversity [1,2] and combinatorial chemistry [3-6] are going to play a major role (Figure 1). Target identification and target validation are now crucial milestones, as the unraveling of the human genome is providing thousands of uncharacterized genes as potential targets for the cure of important diseases. Research laboratories able to identify and validate targets better and faster than competitors will be significantly advantaged, and combinatorial approaches and tools will provide relevant benefits at this stage [7]; nevertheless, the full potential of chemical diversity and combinatorial libraries is evident in the following three steps of the process .

Traditionally the accent in Drug Discovery was put on the throughput, i.e. on the availability of large diversity collections (>>100K), of high-throughput robotics for the handling and the screening of the diversity, and of high-throughput analytical tools for the determination of the structure(s) and of the quality of active compounds. As for the collections, four major sources of compounds are available:

Single compounds (externally acquired or in house prepared);

Natural products from living organisms;

Discrete libraries (parallel synthesis, individual compounds);

Pool libraries (mix and split synthesis, mixtures).

Each source has its advantages and disadvantages, and will be thoroughly examined during the Lecture. Several key messages summarize the current tendencies related to chemical diversity and screening in hit identification:

A collection must contain subsets from all diversity sources, and must evolve by acquisition/synthesis/isolation of novel, relevant individuals or libraries;

Large pool primary libraries are becoming less popular;

Medium-small, high quality, modular discrete libraries are increasingly popular;

Libraries inspired by natural products' complex structures are increasingly popular, especially concerning the so-called chemical genetics approach [8,9].

The second part of this Lecture will present three recent examples referring to lead discovery and lead optimization. The first covers the synthesis of so called "activity profiling libraries", used to determine the nature of proteases in in vitro and in vivo assays and to validate their relevance as targets in Drug Discovery [10]. The second covers modular libraries in solution derived from a common chalcone library [11]. The third [12] reports a high quality solid phase pool library of complex, natural products-like compounds obtained from high quality and yield chemical transformations.

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