Learner Support Services

The University of Bradford Institutional Repository

Editor(s): Kelle, A.

Title: The Changing Scientific and Technological Basis of the CBW Proliferation Problem: A Workshop Report

Project: Bradford Project on Strengthening the Biological and Toxin Weapons Convention (BTWC)

Publication year: 2007

BTWC Science and Technology Reports: No. 7

Publisher: University of Bradford (*http://www.brad.ac.uk*)

Publisher's repository: *http://bradscholars.ac.uk:8080/dspace*

The Changing Scientific and Technological Basis of the CBW Proliferation Problem

A Workshop Report

Edited by Alexander Kelle

February 2007

Table of Contents:

* The workshop on "Preventing the Misuse of 21st Century Chemistry: State of the Art of Drug Development and Delivery, and Selected Enabling Technologies" took place in Belfast on 13 / 14 January 2006 in support of the project funded by a MacArthur research and writing grant on "Preventing the Malign Misuse of 21st Century Chemistry – How to Strengthen the Prohibitory Norm Against Chemical Weapons" (grant no. 05-84295-000-GSS). Additional financial support by the School of Politics, International Studies and Philosophy, Queen's University Belfast is gratefully acknowledged.

Introduction

Alexander Kelle

Historically, the possibilities of waging chemical and biological warfare have been dependent on the state of scientific and technological knowledge in chemistry and biology. To illustrate this point with respect to developments in chemistry, it was a particular aspect of the industrial revolution which made chemical warfare during World War I possible (Aftalion: 2001, 32ff.). As Robinson points out, the "technology initially responsible" for bringing "toxic weapons out from their prehistory" was the "large-scale liquefaction of chlorine gas and its packaging into pressure cylinders" (Robinson: 1998, 18). In the inter-war period civilian research into a new group of organophosphorous compounds in the context of work on plastic additives and fertilizers first led to the development and production of the first nerve agent, Tabun in December 1936. This discovery was followed by the synthesis of Sarin in 1939 and Soman in 1944 (Martinetz: 1995). After World War II civilian work to exploit the new group of toxic organophosphates continued, leading to the development of even more toxic compounds. One of these super-toxic compounds was adopted by the US military and became known as the VX chemical warfare agent during the 1950s (SIPRI: 1971, 71ff.).

Similarly, as Dando has demonstrated for the "three generations of offensive biological warfare programs" of the 20th century, all the military programs were "developing on the back of growth in scientific knowledge." (Dando: 1999, 51) He points out that military BW programs exploited scientific discoveries in the areas of (1) bacteriology, paving the way for the BW-based sabotage activities during World War I (Wheelis: 1999), (2) aerobiology, providing for the knowledge to spread BW agents over large geographic areas, and thereby giving also non-contagious agents their potential to be used as mass casualty weapons, and (3) the beginning biotechnology revolution, which was utilized in the BW program of the former Soviet Union. According to one account, Soviet BW-scientists worked on genetically modified pathogens, including "antibiotic-resistant strains of plague, anthrax, tularemia, and glanders." (Tucker: 1999, 2)

States parties to the Biological Weapons Convention (BWC) have acknowledged the changing S&T basis of the BW proliferation problem in successive quinquennial review conferences, beginning in 1980. While the initial review document statement concerning S&T advances was a rather concise one, BWC states parties since then have seen the need to add more and more developments of relevance to the Convention to the respective paragraphs of the final declarations (Kelle/Nixdorff/Dando: 2006, 43ff.). During the Review Conference's Preparatory Committee meeting in April 2006 BWC states parties agreed to have included among the background documents to be prepared for the Conference one on

> "new scientific and technological developments relevant to the Convention, to be compiled from information submitted by States Parties as well as from information provided by relevant international organisations" (Preparatory Committee for the 6th BWC Review Conference: 2006, 4).

Nine BWC states parties – Argentina, Australia, Czech Republic, Netherlands, Portugal, Russian Federation, Sweden, United Kingdom, and United States – followed this call and submitted such information to the Review Conference Secretariat, which in turn has produced the requested background document (Secretariat of the 6th BWC Review Conference: 2006). This document discusses "significant developments" in the areas of "biotechnology; genomics; proteomics; bioinformatics and computational biology; systems biology; drug discovery, design and delivery; synthetic biology and biological engineering; as well as a number of other relevant developments", such as for example nanotechnology. The 6th BWC Review Conference, however, which was held in Geneva from 20 November to 8 December 2006 broke with tradition and introduced for the first time a catch-all clause, when in its final declaration no specific new areas of concern are mentioned. Instead, the section on Article I states that "[t]he Conference reaffirms that Article I applies to ***all scientific and technological developments in the life sciences*** and in other fields of science relevant to the Convention." (emphasis added; 6th BWC Review Conference: 2006, 10) Unfortunately, this reaffirmation of the comprehensive character of the BWC in relation to S&T advances has not been supported by the establishment of a scientific advisory panel, which some had advocated in the months before the Review Conference. Only a small *Implementation Support Unit* whose tasks are limited to administrative support and improved implementation of Confidence Building Measures has been set up by the Conference. Equally unsatisfactory is the absence of S&T developments from the list of topics to be addressed during the new intersessional process from 2007 to 2010. In

addition, as Pearson has pointed out, "an appeal [contained in the 1996 Final Declaration, AK] to 'scientific communities to lend their support only to activities that have justification for prophylactic, protective and other peaceful purposes, and [to] refrain from undertaking or supporting activities which are in breach of the obligations deriving from provisions of the Convention' has inexplicably been removed" (Pearson: 11 December 2006) from the 2006 Final Declaration. Thus, states parties to the BWC have neither given themselves the organisational structure nor allocated a specific time for reviewing S&T advances between now and the 7th Review Conference in 2011. They have also weakened the normative framework that can serve as guidance for life scientists' actions. Revisiting the issue of codes of conducts as one of the topics to be discussed in 2008 does hardly make up for these omissions.

Similar to the BW control regime, the drafters of the Chemical Weapons Convention (CWC) anticipated changes in science and technology (S&T) and the chemical industry, which is to be inspected under the CWC's industry verification regime. The CWC contains a review mechanism and the Director General of the organisation created to oversee the CWC's implementation – the Organisation for the Prohibition of Chemical Weapons (OPCW) – has been tasked to set up a Scientific Advisory Board (SAB) to advise him on S&T matters of relevance to the CWC. More specifically, the Review Conferences of the CWC, the first of which took place in April/May 2003 and the second of which is scheduled for 2008, are mandated to "take into account any relevant scientific and technological developments." (CWC Article VIII, para 22) In order to do this, the First Review Conference had before it a report by the SAB, addressing a number of S&T areas, most of which were directly related to the day-to-day operation of the CWC. (OPCW: 2003) A farther reaching review of S&T advances was not conducted during the First CWC Review Conference. What has so far become known about preparations for the Second CWC Review Conference to be held in spring 2008, the pattern of the first review process is likely to be repeated. As in the run-up to the first Review an open-ended working group has been set up to prepare the Second Review Conference and the SAB has again started to look into new S&T developments of relevance to the CWC. A meeting to this end involving the International Union of Pure and Applied Chemistry (IUPAC) is scheduled to take place in April 2007 in Croatia.

What is noteworthy in this context is the increasing overlap of areas of concern for both the BW and the CW control regimes. The chemistry of the 21st century is a far cry from the one of the 1980s, which guided negotiations for the CWC verification regime. The new chemistry is utilizing other scientific disciplines and technologies to a much higher degree in its quest for new chemical compounds. Especially in the area of drug development and delivery, scientific and technological advances in biotechnology and genomics, robotics (Vogt: 2002), information technology (Holland and Mitchell: 1999; Ritchie: 2001; Azuaje: 2006 in this volume), and nanotechnology (Davis: 1997; Sahoo and Labhasetwar: 2002; Byrne: 2006 in this volume) act as enablers of combinatorial chemistry and high throughput screening, which in turn have become key tools in pharmaceutical research and development (Wood and Scott: 2000; Wheelis: 2002).

Seen in a wider perspective, it has been acknowledged that we are witnessing a "multidisciplinary technology revolution" (RAND: 2001), what is often overlooked is the fact that many of the products flowing from the biotechnology revolution that will impact on life processes at various levels are basically chemical compounds. All chemical compounds, however, which through their toxic properties could harm man, fall under the prohibitions of the CWC. Some key areas of the revolution in the life sciences threaten to expand not only the range of toxic chemicals that could be misused for malign purposes but also our knowledge of the targets of potentially harmful biologically active chemical substances in the human body. In decades to come, this can be expected to shift the "focus of the proliferation problem from the chemical or biological warfare agent as object of malign manipulation to the physiological target in the human body as the object of attack." (Kelle/Nixdorff/Dando: 2006, 4) Given a plethora of research activities over the last decade, such targets in the nervous, immune and neuro-endocrine immune systems today are much better understood and thus more susceptible to potential misuse. As an NAS study on *Globalization, Biosecurity and the Future of the Life Sciences* has pointed out with reference to microbial threats:

> "While [the] greater awareness of host defense may lead to new strategies for the recognition, prevention, treatment, and prediction of outcome of microbial disease, it also broadens the knowledge base from which bioterrorists could design new forms of biological weapons that disrupt host homeostatic systems, … with diverse and potentially devastating consequences." (NAS: 2006, 37)

The fact that this "paradigm shift is fuelled by the decoding of the human genome and finds its expression in the establishment of new scientific subfields such as systems

biology" (Kelle/Nixdorff/Dando: 2006, 3) leads us back to the various enabling technologies that feed into drug design and development.

At a very general level the genomics and proteomics revolution equals, to use Piers Millett's terms, the discovery of the "dictionaries and thesauri of biology" (Millett: 2007, in this volume). However, already in the year 2000 one survey has cautioned against a "definition creep and the changing meaning of genomics" (Cooke-Degan *et.al.*: 2000) in the reviewed literature. The sub-differentiation of genomics into various subfields becomes evident in another summary, according to which genomic technologies involve "DNA synthesis, sequencing, genotyping, and expression profiling" but also

> "proteomics (peptide synthesis, protein sequencing, and mass spectrometry), and imaging. Innovative technologies might include applications of nanobiotechnology, isolation, imaging, and characterization of single molecules." (Cornell Genomics Initiative, 2001)

To take just one example of the technologies mentioned in this quote, DNA synthesis has over the past few years allowed for the construction of ever longer DNA sequences resulting in the *de novo* synthesis of the Poliovirus in 2002 (Cello: 2002). The possibility to apply such a technology for both benevolent and malevolent purposes, i.e. its dual-use potential, is obvious. Even the resurrection of extinct viruses like the 1918 Spanish flu virus is within the realm of the possible, once the genome of a particular virus has been deciphered.

Although the exact meaning of another term – post-genomics – is still contested, there seems to be general agreement that we live in the post-genomic era. Whether the term is meant to refer to an increasing emphasis on functional genomics (see Millett, in this volume) or to "anything connected with teasing higher biological meaning and function out of raw sequence data" (Science Online: 2006), there seems to be a general recognition that key tasks in this new era encompass the storage, retrieval, analysis, and integration of biological data. This is the domain of bioinformatics. As Francisco Azuaje in his contribution to this volume outlines, this area has moved away from the analysis of single genes and genomes to the complex computational modelling of networks in order to enable the discovery of new drug targets, and the development of new medicines or diagnostic tools. One continuous challenge in this area remains the integration of data from diverse sources and in various formats. (Searls: 2000; Fellenberg: 2003)

Just like bioinformatics, nanotechnology (NT) has over the past decade seen a dramatic increase in research output, funding and recognition as a crucial area of interdisciplinary collaboration involving at least microbiology, bioinformatics, medicine and materials science. Both the integrative dimension as well as the differentiation into several nano-subdisciplines is reflected in the growing number and specialisation of NT-related academic journals. Not only is the number of journals that cover NT across the board increasing – with *Nature Nanotechnology* being one of the more recent additions to the field – the increasing degree of specificity in coverage of some journals is evident as well. Of greatest relevance here are titles like *Nanomedicine*, *Journal of Biomedical Nanotechnology*, and *Nanotoxicology*. The latter journal's mission statement contains a commitment to publish research on "the potential for human and environmental exposure, hazard and risk associated with the use and development of nano-structured materials." As Oberdörster and colleagues point out, nanotoxicology has its roots in studies on ultrafine particles, which have surrounded humans "throughout their evolutionary stages" (2005: 823). Seen from this perspective, nanotechnology is adding just another dimension to the challenge of risk assessment of nanoparticles. While such a risk assessment would address the negative side-effects of nano-engineered materials, it would in all likelihood not cover the dual use dimension of this area of research, i.e. its deliberate misuse for malign purposes. Concerning the dual use potential of nanotechnology an international workshop of the US National Academy of Sciences concluded that because the "technology is expected to make for relatively inexpensive, small scale science, the dual-use risks will be similar to those of more conventional biotechnology in terms of ease of access and detection" (NAS: 2005, 66), i.e. access will be easy, given the to be expected wide-spread diffusion of the technology and, correspondingly, detection of misuse will be difficult, especially given the restrictions of current control mechanisms.

On a different scale than nanoscience and nanotechnology are micro reactors, whose development has made great progress in recent years. Development of micro reactors is likely to have a more immediate impact on chemical production technology than the other S&T developments discussed so far. As Watts explains:

> "Reactions performed in a micro reactor invariably generate comparatively pure products in high yield, when compared to the equivalent bulk reactions, in much shorter times." (Watts: 2007, in this volume)

These characteristics, combined with the possibility to run micro reactors in parallel and thereby scale up the production volume that can be achieved with these devices, can lead to production capacities that might pose a proliferation problem and that also fall under the declaration requirements of the CWC. It is therefore to be welcomed that the OPCW SAB has been tasked to analyse the potential implications of micro reactors for the CWC. This emphasis on micro reactors – but also on nanotechnology – was re-confirmed by the OPCW Director General, Ambassador Rogelio Pfirter, in his address to the 11th Session of the Conference of States Parties to the OPCW (OPCW: 2006, 12).

Yet, no matter how well the SAB or other groups perform assessments of S&T developments that are of relevance to the chemical or biological weapons proliferation problem and corresponding efforts to prevent the spread of such weapons, in the final analysis responsibility to act on such assessments and concomitant recommendations rests with the states parties to the two regimes collectively and on states individually. As a first step, it would be a great improvement if more states were to actively engage in such a technology assessment in the first place. As mentioned above, only nine states parties to the BWC contributed material to a background document on S&T issues for the 6th BWC Review Conference last year. Although there may be a geographical concentration in some areas of cutting edge research described in this report, it is difficult to imagine that less than a dozen states have a stake in the potential regulation of such new areas like DNA synthesis, bioinformatics, nanotechnology, drug development, or micro reactors.

The contributions that follow were commissioned for a workshop that brought together life scientists with CBW arms control and nonproliferation experts. As a result, one of the tasks for the contributors was to provide an overview of their respective areas of expertise in such a way that it would be more easily digestible for non-experts. It is hoped that this approach will make the contributions to this volume of interest to a wider audience. Those wishing to delve deeper into one or more of the areas covered might find the references provided at the end a useful starting point.

Genomics and Proteomics

Piers D Millett

Introduction

> *Dictionaries are like watches; the worst is better than none, and the best cannot be expected to go quite true.* ~ Samuel Johnson

In many ways, genomics and proteomics are the dictionaries and thesauri of biology. The databases which house the information generated by the two disciplines contain descriptions and functions of the 'words' of life. The biological alphabet may consist of only four letters but this by no means seems to limit the diversity of both form and function. This paper briefly reviews the current status of these two rapidly developing fields and discusses their potential for finding non-peaceful applications.

Genomics

The life sciences have witnessed many important events over the last couple of decades, not least of which is the advent of genomics. Lockhart *et al* have noted that:

> …the massive increase in the amount of DNA sequence information and the development of technologies to exploit its use… *[have prompted]* new types of experiments… observations, analyses and discoveries… on an unprecedented scale…Unfortunately, the billions of bases of DNA sequence do not tell us what all the genes do, how cells work, how cells form organisms, what goes wrong in disease, how we age or how to develop a drug… The purpose of genomics is to understand biology not simply to identify its component parts. *(Lockhart and Winzeler: 2000).*

Four interlinked areas of interest have emerged from these efforts: **genome mapping and sequencing** to locate genes on the chromosomes (Boguski and Schuler: 1995) and determining the exact order of the nucleotides that make up the DNA of the chromosomes (International Human Genome Sequencing Consortium: 2001); **structural genomics** *to provide…* [a] *model structure for all of the tractable macromolecules that are encoded by complete genomes* (Bremner: 2001); **functional genomics** to *flesh out the relationships between genes and phenotypes* (White: 2001); and **comparative genomics** to identify functional sequences by comparing various genomes (Bofelli, Nobrega and Rubin: 2004).

Genome Mapping and Sequencing

There are two general types of map – genetic maps and physical maps. Genetic maps locate genes in relation to genetic markers, often restriction fragment length polymorphisms (RFLPs), variable number of tandem repeat polymorphisms (VNTRs), Microsatellite polymorphisms, or single nucleotide polymorphisms (SNPs). Genetic maps can be used to create the scaffold for more detailed physical maps. Physical maps order clones and/or landmarks to isolate and position genes. This facilitates the study of genome organisation and evolution and forms a useful framework for genome sequencing. Three types of physical map are commonly used: radiation hybrid maps (landmark-only maps) (Cox *et al*: 1990); clone–based maps (Birren, Mancino and Shizuya: 1999); and sequence-ready contiguous maps (Marra *et al*: 1997) (Gregory *et al*: 1997).

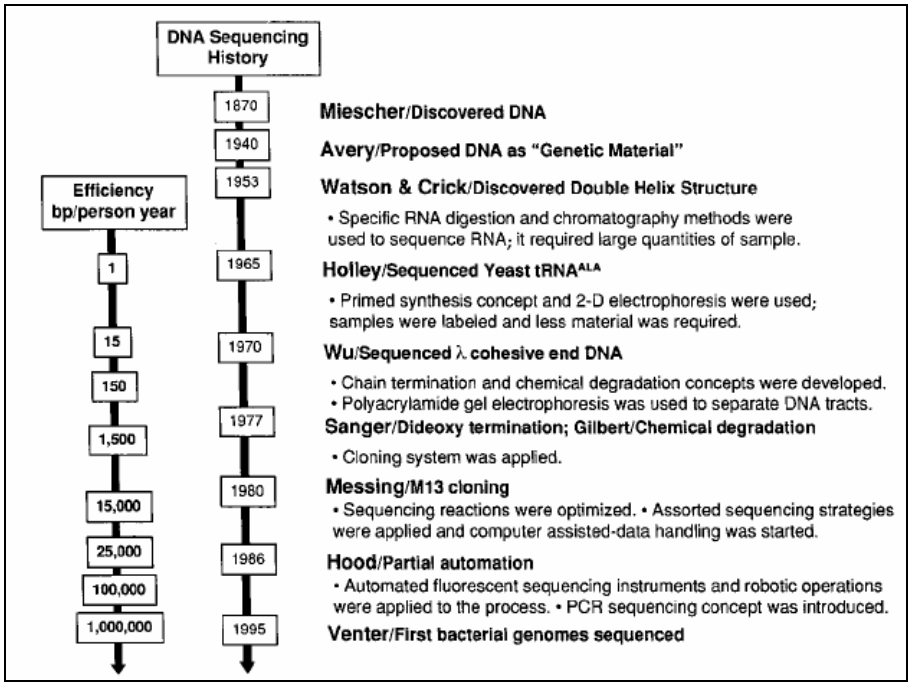


FIGURE 1: A Brief History of DNA Sequencing. (Messing and Llaca: 1998)

Once mapped, genes can be sequenced by identifying the order of their nucleotides. As of 22 August 2005, the three international depositories of gene sequence information (EMBL Data Library of the European Molecular Biology Laboratory *et al*) reported having passed 100 gigabases of sequence from 165,000 organisms (Anon (online): 2005). Of particular importance were the publication of the first eukaryotic genome (a yeast) in 1997 (Clayton *et al*: 1997), the first animal genome (a worm) in 1998 (The C.elegans Sequencing

Consortium: 1998), and the human genome (Venter *et al*: 2001; Lander *et al*: 2001) in 2001.

An overview of key events in the development of the technology to enable these breakthroughs can be found in Figure 1. Currently the most utilised methodology is fluorescent DNA sequencing (Wilson and Mardis: 1999).

Structural Genomics

One approach to attempt to contextualise genes is to examine the structure of its product. This offers insights into its function. Relationships between genes, which are difficult to obtain through sequence analysis, can be more readily characterised because structural traits are better conserved over time. Structural genomics, which is closely related to proteomics, takes advantage of many of the same tools as traditional structural biology but has developed a different motivation, automation and scale (Bremner: 2001). Unfortunately much of the information gained provides details of molecular function and does not usually confer a detailed understanding of its cellular role. For this more systemic approaches are necessary.

Functional Genomics

Functional genomics contextualises genes by adopting such a systemic approach - focusing on all the genes in a genome rather than characterising a single gene at a time. Three areas of functional genomics of particular note are: *interfering with gene function* (Figure 2)*; gene-expression profiling* (Hughes *et al*: 2000)*; and genetic mapping of quantitative trait loci* (Van Eerdewegh *et al*: 2002).

Core questions which functional genomics attempts to answer include *when is a gene expressed, where is its product localized, with which other gene products does it interact and what phenotype results if a gene is mutated* (Steinmetz and Davis: 2004). This provides the possibility of elucidating the genetic variants for complex traits. Due to its systemic approach and the technologies it uses, functional genomics is closely related to systems biology.

TARGETED DELETION BY HOMOLOGOUS RECOMBINATION	INSERTIONAL MUTAGENESIS	RNA INTERFERENCE
Precise gene deletion can be readily achieved by homologous recombination in yeast and mouse. Because this approach removes the targeted gene, functional reduction is complete. In organisms in which it works, this method is the gold standard. Unfortunately, homologous recombination does not work efficiently in several model organisms, including *Arabidopsis* and *Caenorhabditis elegans*. Although it has been shown to work in some cases, as seen recently in *Drosophila*, the efficiencies are still too low for systematic application.	Disruption of gene sequences can be achieved by insertional mutagenesis using transposons or other insertion sequences. Because the genome insertions are random, screening for disruption in a gene of interest is required. The insertion can lead to complete, incomplete or no functional reduction, depending on where the integration occurs. The insertion site and level of functional reduction therefore need to be determined experimentally. The method has been used extensively in *Arabidopsis* and *Drosophila*, yeast, mouse and *C. elegans*.	RNA interference (RNAi) is the newest technology for reducing gene expression. It follows reports of gene silencing in plants and other model organisms, and is based on the observation from *C. elegans* that adding double-stranded RNA (dsRNA) to cells often interferes with gene function in a sequence-specific manner. In most cases, the level of functional reduction is incomplete and the level of specificity is not entirely predictable. Nevertheless, RNAi has been shown to work in many model organisms. Current applications are primarily in *C. elegans*, *Drosophila*, various plants, tissue culture cells of *Drosophila* and mammals.

FIGURE 2: Comparison of Knock-Out Approaches (Adapted from Steinmetz and Davis: 2004).

Comparative Genomics

By comparing the sequences from different genomes, it is possible to identify areas which are conserved and as a result which may be functionally important. There is value in comparing species which are closely related (Bofelli *et al*: November 2004) as well as those which are more distant (Bofelli, Nobrega and Rubin: 2004). Using closely related species offers the possibility to identify a larger number of functional elements but makes them more difficult to identify. Conversely, using species more evolutionary distant reduces the number of functional elements they share but makes them easier to identify. Being able to extrapolate from simpler, better understood organisms (such as the mouse) into more complex ones (such as humans), also has benefits for functional genomics. It has also recently become feasible to compare the genomes of different members of a single species in an attempt to be able to identify functional sequences which are more species specific (Bofelli *et al*: November 2004).

Features compared include: sequence similarity, gene location, the length and number of exons, the amount of noncoding DNA in each genome, and highly conserved regions. This work is, of course, done on computers. The most commonly used tool is Basic Local Alignment Search Tool (BLAST) made available by the National Center for

Biotechnology Information in the USA. BLAST can be used to infer functional and evolutionary relationships between sequences as well as to help identify members of gene families.

Proteomics

Patterson and Aebersold defined proteomics as:

> the systematic study of the many and diverse properties of proteins in a parallel manner with the aim of providing detailed descriptions of the structure, function and control of biological systems in health and disease *(Patterson and Aebersold: 2003)*.

Proteins are not only involved in the majority of all biological processes but collectively contribute significantly to the understanding of biological systems. Proteomics assesses a range of variables, including: sequence; quantity; state of modification; interactions with other proteins; activity; and subcellular distribution and structure.

Proteomics – Basic Concepts

Proteomics evolved from studies of traditional protein chemistry. In combination with genomics, protein chemistry provided a link between the observable function of a protein and the gene that encoded it (Figure 3). The advent of large repositories of gene sequences further facilitated protein identification by allowing correlation of sequences derived experimentally through protein chemistry to sequences in the databases. The development of genome databases opened the watersheds for this approach. Protein identification was now primarily restricted by capacities to extract sequence information from proteins and correlate this with the information in the databases. As a result proteomics was dependent upon the development of technologies to provide the necessary resources.

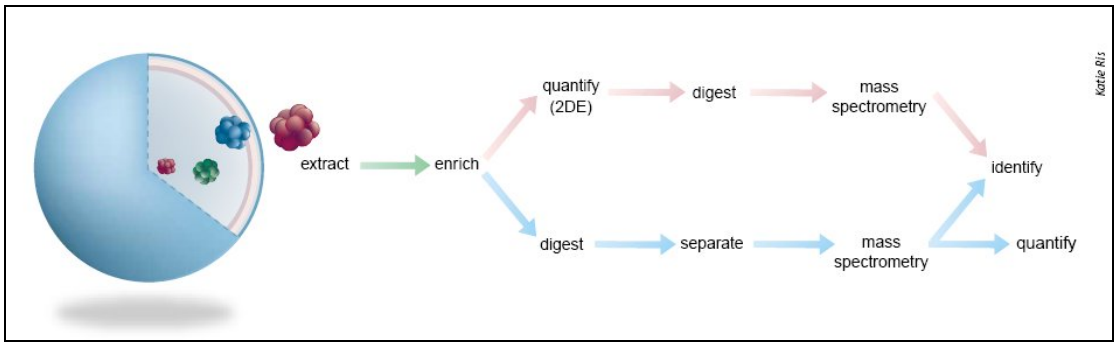


FIGURE 3: Quantitative Protein Analysis from the Cell to the Identified Protein. (Patterson and Aebersold: 2003).

Proteomics Tools

Many different technologies have been and are being developed to facilitate proteomics. A variety of existing and prototype technologies were described in March 2003 in a supplement to *Nature Genetics* (see Figure 4).

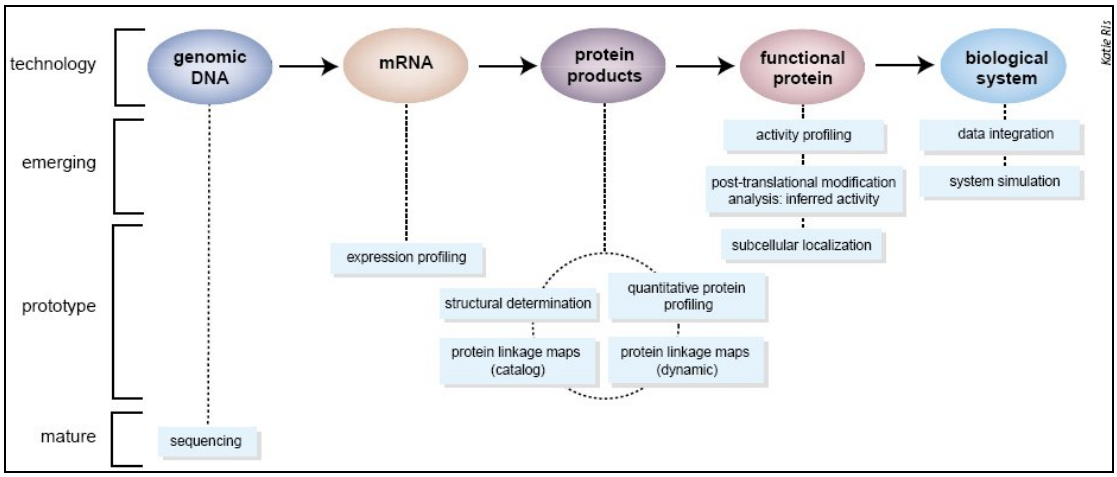


FIGURE 4: The Current Status of Proteomic Technologies (Patterson and Aebersold: 2003).

It was the confluence of these and other technologies in the mid-1990s which led to creation of proteomics (Figure 5).

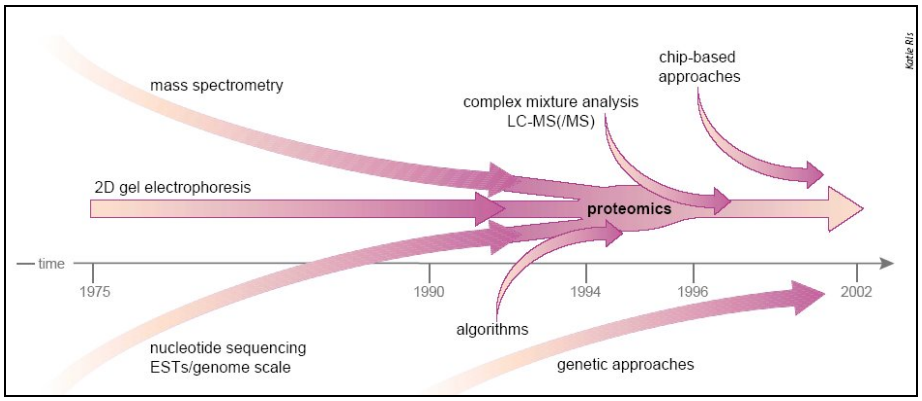


FIGURE 5: The Confluence of Divergent Technologies leads to the Advent of Proteomics.

Proteomics Applications

Speicher asserted that proteomics has an *Infinite Potential* (Speicher: 2002). Although this rather limits what it is and is not possible to do in this section, some thought has been given to how the coming of age of this science may impact upon the fight against disease. Sam Hanash of the University of Michigan suggests that proteomics will facilitate:

> "The characterization of submicrobial proteomes (for example, secreted proteins, surface proteins and immunogenic proteins)
> Comparative analysis of different strains
> Comparative analysis of different physiological states
> Identification of proteins related to pathogenicity
> Identification of proteins involved in host-pathogen interactions
> Evaluation of mechanisms of action of antimicrobials."(Hanash: 2003)

Hanash goes on to provide an example of one successful application of proteomics to combating disease. It is asserted that comparative proteomic studies of the parasite *Plasmodium falciparum* have already led to novel drug and vaccine targets.

Potential Malign Applications

It is possible scientific advances can find detrimental or malign applications in addition to those beneficial to humanity. The relationship between science and warfare is well documented. In the case of the life sciences, it is possible that they could be used for the acquisition or use of chemical, biological or toxin weapons. The prohibition against these weapons can be traced back throughout human development. Today, this proscription is embodied in the 1972 Biological and Toxin Weapons Convention and the 1993 Chemical Weapons Convention. These international instruments, which not only binds the action of their member States but also every individual in those States, prohibits the development, production, acquisition, transfer, stockpiling and use of chemical, biological or toxin weapons.

The first article of the BWC details part of the scope of this prohibition:

> never in any circumstances to develop, produce, stockpile or otherwise acquire or retain:
> (1) Microbial or other biological agents, or toxins whatever their origin or method of production, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes;
> (2) Weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict. (United Nations General Assembly: 1971)

Similar language exists in the Chemical Weapons Convention. Thus under international law, any activity which cannot be justified for prophylactic, protective or other peaceful purposes is already prohibited.

In practice, scientific research and development in the life sciences has both peaceful and malign applications. This creates a nuanced situation, once necessitating a balancing between costs and benefits. Some advances will be of more use to anyone wishing to acquire or use chemical, biological and toxin weapon. Identifying activities which pose the greatest proliferation risk is a difficult issue and one still in the process of being addressed (See for Example: The National Academies: 2004). It has been suggested that one conceptual starting point for such efforts is to differentiate between directly applicable advances and enabling advances (Millett: 2005). Certain developments have a specific application which limits their potential utility – such as an ability to influence the production of a single hormone. Other developments have a virtually unlimited range of application and are innately understood to enable other activities, such as the elucidation of a new method of synthetically producing very complex biological samples. Characterising advances in this manner allows a very simple cost/benefit analysis to be undertaken – the benefits of enabling advances to furthering the aims of science will undoubtedly outweigh their proliferation potential. As a result, it has been suggested that enabling advances should be exempted from any regime to regulate scientific activity.

It is clear from the discussions above that both genomics and proteomics are enabling advances. The sheer scope of the disciplines and diversity of their applications makes it very difficult to discuss their potential malign applications which any specificity. However, for illustrative purposes, a recent development is reviewed and a potential malign use proposed.

In the September 2004 edition of the *Journal of Biomolecular Screening*, Huff *et al.* described a label-free protein screening technology (Huff *et al*: 2004). It makes use of an atomic force microscope to detect proteins and pathogens. The micron-scale tip of the atomic force microscope is used to scan and amplify the samples topographical features. This allows it to detect molecules bound to proteins by detecting nanometre changes in height. In addition, coating the tip with biological molecules provides the opportunity to bind specific proteins and can allow measurements of intermolecular binding. The authors used this concept to develop a label-free, multiplexed, chip-based detection system that can directly determine the amount of virus particles in a sample.

This tool will have obvious applications in peaceful scientific research – it will potentially facilitate the assessment of virus titre in patient samples as well as assisting in numerous high-throughput screening processes in modern drug-discovery techniques. It would also have potential for both defensive and offensive application in relation to

chemical, biological and toxin weapons. In a defensive programme, it could eventually be developed into a rapid detection system for chemical, toxin and biological agents. Alternatively, in an offensive programme, the same development has the potential to facilitate the identification of novel toxins, in developments tests to assess agent concentrations, the efficiency of potential countermeasures and progress in attempting to counteract them.

Conclusions

This paper provides a brief overview of the disciplines of genomics and proteomics and has asserted that whilst that it is worth noting that they do have potential applications for hostile or non-peaceful purposes, due to their overwhelming potential to benefit mankind their development should be pursued to the fullest extent possible. It should be noted that although every attempt has been made to make this paper as exhaustive as possible, there are many additional elements worthy of note, such as a consideration of chip-based micro-arrays. There is also considerable scope for overlap with other areas under consideration in other contributions to this volume, such as systems biology and bioinformatics.

From Genes and Genomes to Complex Systems for Supporting Knowledge Discovery

Francisco Azuaje

This paper provides an overview of some critical, recent advances in bioinformatics, with an emphasis on applications and potential implications for the development of systems biology. It also discusses current technological and policy requirements and challenges necessary for supporting a more open and integrated international scientific community.

The study of biological functions and phenomena has shifted from the analysis of single biological entities (e.g. genes, proteins) to collections of several thousands of these entities interacting via complex information processing networks.

The area of bioinformatics was traditionally associated with the application of information and communication technologies to support molecular biology research. After the completion of several genome sequencing projects it has become a fundamental component of the knowledge discovery process. Such a process includes the identification of new diagnostic and therapeutic approaches. In general, bioinformatics currently aims to integrate a wider spectrum of technologies and disciplines to support and integrate *in silico*, *in vitro* and *in vivo* experimental methodologies and findings. Bioinformatics is constantly evolving with the emergence (or promise) of a *systems biology* in the post-genome era. The expression systems biology is currently used to refer to the integration of multiple types of data and information sources, research teams and predictive modelling techniques to describe and explain the behaviour of biological systems at different organisational and complexity levels.

Thus, bioinformatics has become a discipline of integration, which is required for the development of systems biology: Integration of skills, organisations, data and information sources, techniques and analytical views. Current advances range from the description of single genes, small interaction circuits and larger networks of genes and proteins. Such advances require the application of several computational and mathematical approaches ranging from probabilistic simulations and relatively complex sets of differential equations through simulations of networks in which genes (or proteins) are seen as connected switches to larger, higher level dynamic representations of pathways. These studies aim to describe the behaviour of genes and proteins over time (e.g.

concentrations), dynamic (time-dependent) relationships between relatively small groups of genes and proteins, as well as the representation of the flow of information in more complex networks of interacting entities and in modular, hierarchical views of specific networks.

Recent advances in bioinformatics have also contributed to the revision of the concept of *gene or protein function*. Thus, biological function may be investigated through the definition of networks of biological entities (e.g. genes and proteins) based on experimental and computational methods. The inference, analysis and integration of networks of genes and gene products are fundamental goals of post-genomic research. Over the past few years several data- and knowledge-driven computational approaches have been proposed, which have provided relevant insights into the informational complexity and function of some of such networks in different organisms. In general, a node in such networks represents a biological entity and a link represents a functional interaction between a pair of entities (e.g. gene regulation, physical protein-protein interaction, etc.). The mathematical analysis of the resulting networks in different model organisms and biological phenomena also provides the basis for understanding important functional properties of these systems, e.g. interconnectivity, robustness, evolution, specialisation. This offers new opportunities for detecting potential therapeutic targets, understanding evolution and re-engineering systems. These are fundamental components of an "information paradigm" necessary to understand both normal and disease phenomena and conditions.

Borrowing a metaphor from the world of gastronomy: the sequencing of genomes may be seen, for example, as the definition of the list of ingredients required to prepare a meal. It is not, as the media have commonly argued, the construction of a "blue print" of systems or organisms. Systems biology would provide the next steps required to understand *how* and *when* such "ingredients" should be combined in order to explain or manipulate the resulting or desired biological outcomes. Following this metaphor: the modelling processes described above would provide different resolutions, levels of details or views in the description of the recipes required to understand the function (and malfunction) of the systems under study.

In practice, how can bioinformatics exploit pathway *network-based knowledge* to support the development of new drugs, vaccines and diagnostic tools? One of the possible approaches comprises the application of *comparative genomics* principles. Comparative

genomics has traditionally aimed at identifying structural (sequence-based) similarities between groups of genes or proteins. A network-based approach aims to identify fundamental functional and structural features distinguishing, for example, hosts and pathogens on the basis of computational representations of their molecular pathways. One major challenge is to detect subtle, but relevant, differences between their functional pathways, i.e. networks implementing processes. This is a complex problem as there are significant similarities between human and many pathogens in terms of metabolic pathways, for instance. In the case of infectious diseases a key goal is then to detect differences between pathogens, vector organisms and hosts in the hope that such differences will highlight potential drug targets (e.g. antibiotics) that will kill the pathogen while significantly reducing negative effects on the host (e.g. humans). Based on this assumption, traditional targets have included metabolic pathways and other key networks in pathogens that substantially differ from those implemented by humans (Chaudhary and Roos, 2005). For instance, with regard to humans, many parasites have developed alternative strategies to synthesise essential proteins. Some parasites (e.g. Leishmania and the parasite causing Chagas disease) exhibit metabolic pathways that are not present in humans. Many pathogens display significant differences in connection to the metabolism of amino acids, others exhibit alternative pathways to compensate for loss of function, and others (e.g. the malaria parasite) feed from some of the host's resources (e.g. haemoglobin) to survive. These differences may point out potential drug targets to fight infections (Chaudhary and Roos, 2005).

Advanced software tools are required to identify and analyse structural and functional differences between pathway networks. In this direction recent research has provided promising evidence for the potential of comparative analysis of networks of protein interactions relevant to infectious diseases. LaCount and colleagues (2005), for instance, experimentally inferred a large-scale version of the network of protein-protein interactions of the parasite causing the most severe type of malaria (*Plasmodium falciparum*). They then performed bioinformatics analyses of the topology of this network (i.e. connectivity patterns) and other functional properties (e.g. gene co-expression and available validated functional annotations for the proteins) to detect groups of proteins involved in important processes relevant to this disease (e.g. gene transcription, invasion of host cells, etc.). This study reported significant similarities (conserved patterns) of specific network regions between *P. falciparum* and other model organisms, i.e. *Saccharomyces cerevisiae* (baker's yeast), *Drosophila melanogaster* (fruit fly), *Caenorhabitis elegans*

(nematode worm) and *Helicobacter pylori.* The resulting networks comprise dense interconnected structures which are significantly shared by yeast and worm. However, some of such sub-networks were not detected in *P. falciparum*. Furthermore, the authors provided strong statistical evidence to reject the hypothesis that all such differences were mainly caused by noise or experimental bias of the available data. Another key conclusion of this study is that "conservation of specific groups of related genes does not necessarily imply conservation of interactions among their encoded proteins" (LaCount *et al.*, 2005). These types of findings at least motivate further research on the application of computational and mathematical approaches to determine potential network-based drug targets.

Other researchers are currently exploring the possibility of analysing properties such as *informational robustness* of systems to identify potential drug targets or therapeutic strategies. For example, it has been suggested that the induction of dormancy in tumour cells, instead of trying to eradicate the tumour, may be a clinical strategy for treating cancers (Kitano, 2004a). This may be achieved by detecting architectural, topological and dynamic features defining the robustness of a particular biological system to particular perturbations. Similar strategies have been proposed for the treatment of the HIV infection (Kitano, 2004b).

The post-genome bioinformatics era is also characterised by and dependent on the development of software tools and infrastructures to allow researchers to access, combine and analyse geographically- and computationally-distributed information (Global Grid Forum, 2005; grid.org, 2005). Its success will be driven by our capacity to propose and promote standards for representing, storing and sharing experimental data and analysis results. It will also require the implementation of secure and user-friendly computational platforms for multi-disciplinary communities. Such advances will significantly support emerging technologies, such as *Grid computing*, that have the potential to significantly accelerate scientific research and drug design. Through Grid computing scientists would be able to use a massive *virtual computer* with the capacity to access multiple resources (machines, databases, programs, etc.) in a unified, automated and transparent fashion.

Other important factors for the development of bioinformatics and systems biology are *Open Source* (OSI, 2005) software and *Open Access* publishing (Butler, 2004; Worlock, 2004). The former does not only refer to the availability of free software tools and access to their source codes, but also it implies other crucial issues relating to software distribution terms. For example, an *open source license* allows modifications to the

original software and requires that the distribution of the derived products be done under open source terms. Such licenses should not discriminate people, communities or organizations. Moreover, it should not restrict other software products (including close source software) that may be used or distributed with the licensed software tool. Open source systems also aim to produce computing platform- and interface-independent software. Open access publishing ensures that scientific articles and related contributions are freely accessible via the Internet and stored in international open access repositories (e.g. www.pubmedcentral.nih.gov). With this publication method the authors grant to any user the right to reproduce, use and distribute a research article in any form if there is proper attribution of authorship and correct citation, and if there is no introduction of major errors to the original information. Representative examples of open access publishing initiatives are: *BioMed Central* (www.biomedcentral.com) and the *Public Library of Science* (www.plos.org). The traditional publication industry has also started to explore the feasibility of open access. For example, the *Oxford Open* initiative (www.oxfordjournals.org/oxfordopen) currently include full open access to some of the Oxford University Press journals (e.g. Nucleic Acids Research) and optional open access modalities (e.g. Bioinformatics).

Open source software and open access publishing may play a fundamental role in the promotion of standardized, transparent, integrated and global research and development resources. From ethical and security perspectives, it has been suggested that both open source and open access publishing may result in opportunities to foster scrutiny, monitoring and a more effective peer-review process of methodologies, systems and applications in a more integrated, international environment. On the other hand, some users, policy makers and researchers may suspect that such openness (software source and access publishing) and integration (global distributed computing, e.g. Grid technologies) may promote the uncontrolled proliferation of technologies, knowledge and materials, which may be misused or illegally manipulated (e.g. terrorism). However, many of us believe that the potential benefits of open source and open access publishing will outweigh potential scientific or security threats. Moreover, they offer opportunities to regulate, reduce or prevent potential hazards or risks through the improvement of our knowledge of how to deal with possible negative impacts and of how to promote global development, safety and cooperation.

In conclusion, recent advances in bioinformatics toward the development of systems biology not only offer new technological opportunities, but also ethical and legal challenges. We may be able to meet the requirements of system biology in a safer and integrated world by supporting reliable access to multiple types of data resources, programs and applications. This will allow scientists to study biological phenomena at different levels of biological complexity and systemic organisation. This ability will only be possible through the implementation of a global and cooperative infrastructure for sharing, annotating, analysing and validating methods and findings. Such an infrastructure requires the development of standards and protocols to encourage and facilitate controlled and shared vocabularies, data formats and usage policies.

Nanotechnology and Nanomedicine

J. Anthony Byrne

Introduction to Nanotechnology

There are many different definitions of what nanotechnology is, or indeed, isn't. One of the most wide ranging and all encompassing definitions was given by the US National Science and Technology Council (NSTC: 2000). Accordingly, nanotechnology is:

> "Research and technology development at the atomic, molecular, or macromolecular levels, in the length of approximately 1–100 nm range, to provide a fundamental understanding of phenomena and materials at the nanoscale, and to create and use structures, devices, and systems that have novel properties and functions because of their small size. The novel and differentiating properties and functions are developed at a critical length scale of matter typically under 100 nm. Nanotechnology research and development includes integration of nanoscale structure into larger material components, systems, and architectures. Within these larger scale assemblies, the control and construction of their structures and component devices remain at the nanoscale".

The concept of nanotechnology is attributed to Nobel Laureate Richard Feyman due to a lecture he gave in 1959 "There is plenty of room at the bottom" which was subsequently published in 1960. (Feynman: 1960). Feynman talked about storage of information on a very small scale, writing and reading in atoms, miniaturisation of the computer, building tiny machines, building tiny factories and about building electronic circuits with atoms.

> "In the year 2000, when they look back at this age, they will wonder why it was not until the year 1960 that anybody began seriously to move in this direction." (Feynman: 1960)

Feyman's ideas were well ahead of his time and indeed he did not use the term 'nanotechnology' to describe his ideas. The first use of the word 'Nanotechnology' has been attributed to Norio Taniguchi in a paper published in 1974 (NSTC: 2000). In 1986 Eric Drexler published his book "Engines of Creation" in which he describes his ideas of molecular nanotechnology used to build miniature machines and devices from the bottom up using self-assembly. Many scientists from mainstream disciplines, Biology, Chemistry

or Physics will argue of course that they are and have been 'doing' nanotechnology for years and that it is nothing new. Indeed chemists play with atoms and molecules which are sub-nano and molecular biology deals with the understanding and application of biological nano-scale components. However, nanotechnology as described by Drexler is not merely using nano-components but rather nanotechnology is a coming revolution which will affect us in every aspect of our lives. Nature has used nanotechnology for millions it has taken millions of years to develop this nano-technology by a process of evolution and natural selection. Nanotechnologists not only wish to mimic how nature does this but we want to make nano-devices to do much needed tasks better than and faster than nature can. Nanotechnology is an emerging research field which promises to have a wide range of interesting applications. By definition it encompasses all technology that aims to create nanometer-scaled structures or is able to address or manipulate matter at the nanometer level.

Roughly nanotechnology can be divided in two main parts: top-down and bottom-up. Development in the top-down methodology was mainly driven from the traditional disciplines of (electrical) engineering and physics, while the bottom-up approach, starting from single atoms and molecules has more affinity with chemistry and molecular biology. Nanotechnology plays at the interface of these two approaches and is therefore inherently multidisciplinary.

Just imagine that in the future we will be able to produce nanoscale machines or devices which can manipulate atoms and molecules to build functional structures or devices, or to do basically anything we choose? To be able to do this would indeed create a complete technological revolution. Of course this would affect not only our quality of life but there are many ethical and social issues which would need to be addressed. If we could build such nano-scale devices they would need to be controlled. What if the programming went wrong either by accident or by design. Instead of having technology to build and create we could have a weapon of mass destruction which could be under the control of extremists or indeed under the control of the device's themselves. This ethical issue has indeed already generated a growing "anti" nanotechnology movement. There are an increasing number of nano-fiction books being published which depict the end of the World caused by the release of nanotechnology on the earth (and even on the moon). However, keeping our nerve we can at least begin to imagine what might be possible. Imagine that we could design nanobots which could be injected into the bloodstream (or even make their way through the skin). These "nano-meds" are programmed to seek out

and repair tumour cells. Or they may be programmed to repair a dysfunctional organ, or they may be programmed to turn off the biological clock for cell death, or repair ageing tissue. These ideas may be nano-fiction today but already billions of pounds are being poured into research towards such objectives.

Nanomedicine

In Feynman's famous lecture indeed he mentions the possible use of nanodevices in medical applications;

> "A friend of mine (Albert R. Hibbs) suggests a very interesting possibility for relatively small machines. He says that, although it is a very wild idea, it would be interesting in surgery if you could swallow the surgeon. You put the mechanical surgeon inside the blood vessel and it goes into the heart and ``looks'' around. (Of course the information has to be fed out.) It finds out which valve is the faulty one and takes a little knife and slices it out. Other small machines might be permanently incorporated in the body to assist some inadequately-functioning organ."
>
> (Feynman: 1960)

We have not yet reached the point at which Albert Hibbs' idea of micro or nano-surgery is reality, however, medical applications of nanotechnology are probably those with the most exciting potential. Indeed, interest in the medical applications of nanotechnology has led to the emergence of a new field called nanomedicine. Nanomedicine has been described as the process of diagnosing, treating and preventing disease and traumatic injury, relieving pain and preserving and improving human health, using molecular tools and molecular knowledge of the human body. In short, nanomedicine is the application of nanotechnology to medicine (NSTC: 2000).

Nanomedicine is a large area and includes nanoparticles that act as biological mimetics, 'nanomachines', nanofibres and polymeric nanoconstructs as biomaterials (e.g. molecular self-assembly and nanofibers of peptides and peptide-amphiphiles for tissue engineering, shape-memory polymers as molecular switches, nanoporous membranes), and nanoscale fabrication-based devices (e.g. silicon microchips for drug release and micromachined hollow needles and two-dimensional needle arrays from single crystal silicon), sensors and laboratory diagnostics. Research into the delivery and targeting of pharmaceutical, therapeutic, and diagnostic agents using nanosized particles is as the forefront of nanomedicine (NSTC: 2000). Freitas (2005) gave a partial taxonomy of nanomedicine technologies which was broken down into 18 classes (see Table 1) and 96

sub-classes. This gives us some idea of the potential for applications of nanotechnology in medicine.

Table 1 Main classes of nanomedicine technologies (from Freitas: 2005)

Raw nanomaterials	DNA manipulation, sequencing, diagnostics	Molecular medicine
Nanostructured materials	Tools and diagnostics	Artificial enzymes and enzyme control
Artificial binding sites	Intracellular devices	Nanotherapeutics
Control of surfaces	BioMEMS	Synthetic biology and early nanodevices
Nanopores	Biological research	Biotechnology and biorobotics
Cell simulations and cell diagnostics	Drug delivery	Nanorobotics

Nanotechnology in drug delivery

While nanomedicine may be hailed as revolutionary and generate great excitement, most working in the fields of drug development and drug delivery will recognize that many nanomedicine technologies are not really new. In fact many are already used to treat patients. Indeed, since 1990, many drugs referred to as nano-therapeutics have been approved as products for clinical use. Most are anticancer drugs i.e. liposomes (e.g. DaunoXome), polymer coated liposomes (Doxil, Caelyx), polymeric drugs (Copaxone), antibodies (Herceptin, Avastin) and antibody conjugates (Mylotarg), polymer-protein conjugates (Oncaspar, Neulasta) and lately, a nanoparticles containing paclitaxel (Abraxane). These are nano-scale and commonly multicomponent constructs and can be viewed as the first nanomedicines to bring clinical benefits. So these nanomedicines are not really new. Many of these concepts e.g. antibody conjugates, liposomes, nanoparticles, and polymer-conjugates were developed in the 1970s (NSTC: 2000). However, significant funding has been given over to the research and development of nanotechnologies that have great potential to contribute to medicine in the short and longer term. Today, nanotechnology and nanoscience approaches to particle design and formulation are beginning to expand the market for many drugs and are forming the basis for a very

profitable niche within the pharma industry, but some predicted benefits are hyped (Moghimi, Hunter and Murray: 2005).

However, exemplars in the scientific literature which demonstrate the potential and the reason for excitement surrounding nanomedicine are overwhelming. The reader is referred to the recent reviews by Freitas (2005) and Moghimi, Hunter, and Murray (2005) for an overview of the current 'state of the art' in nanomedicine and nanotechnology in drug delivery.

Nanomedicine today has branched out in hundreds of different directions, but the core concept remains that an ability to structure materials and devices at the molecular scale can bring enormous immediate benefits in the research and practice of medicine. It has been proposed that miniaturisation of medical tools will provide more accurate, more controllable, more versatile, more reliable, cheaper, and faster approaches to enhancing the quality of human life (Freitas: 2005).

Risks of Nanotechnology

Nano-fiction books have generated much concern over the possible risks of nanotechnology. Indeed Prince Charles has been quoted by the Press for raising fears about the apocalyptic future brought upon us by nanotechnology.

> "The Prince is said to fear a worst case scenario in which nanotechnology spin-offs would annihilate life on earth.
>
> The Prince is said to be concerned that this could lead to an apocalyptic 'grey goo' theory in which these miniscule robots would reproduce like viruses, feeding off all natural matter and consume the whole planet; leaving behind only a grey goo."
>
> Scotland on Sunday, 27 April 2002

In 2003 the Prince again spoke out about his concerns but this time with a more sober approach calling for developments in nanotechnology to be used wisely and appropriately.

However, the Prince is not the only one to be concerned about the potential risks of nanotechnology. Professor David Williams, University of Liverpool, is a member of the Scientific Committee on Emerging and Newly Identified Health Risks of the European Commission. This committee has presented their report on nanotechnology for adoption (NSTC: 2000). Williams recently published an article entitled 'The risks of nanotechnology' (2005). He cautioned that exploiting the technology for the benefit of

mankind may expose the same humans to new health risks. His main concerns were to do with the lack of knowledge concerning the toxicology of nanoparticles when inside the human body.

In July 2004 the Royal Society published a report entitled "Nanoscience and nanotechnologies: opportunities and uncertainties" Royal Society: 2004). The report illustrates the fact that nanotechnologies offer many benefits both now and in the future but that public debate is needed about their development. It also highlights the immediate need for research to address uncertainties about the health and environmental effects of nanoparticles – one small area of nanotechnologies. It also makes recommendations about regulation to control exposure to nanoparticles.

Conclusion

The concepts of nanotechnology have far reaching implications yet at the moment some of these concepts seem like science or nano-fiction to us. However, millions of pounds are being poured into research and development of nanotechnology which has the potential to change our world forever. There are risks associated with the unknown and as scientists we have a responsibility to protect ourselves, those around us, and the world we live in.

> "Although many people believed that nanotechnologies will have an impact across a wide range of sectors, a survey of experts in nanotechnologies across the world identified hype (misguided promises that nanotechnology can fix everything) as the factor most likely to result in a backlash against it."
>
> (Royal Society Report: 2004)

Drug design, combinatorial chemistry and drug development

Isabel Rozas

Introduction

There are many drugs in the market and around 6,000 chemical compounds are the bases of them. Therefore, one could consider that there is no need for more drugs. However, if we look at diseases, we can see that there are still many without a decisive treatment, including cancer, Alzheimer's or Parkinson's. Besides, new diseases appear as occurred with AIDS in the 1980s and Bovine Spongiform Encephalopaty (BSE) in the 1990s and the avian flu during 2005. Moreover, there is a problem with uncommon diseases: those that affect less than 200,000 people as happens with Tourette's syndrome or the Paget disease. The search for new specialities is therefore totally justified.

Research in new drugs is the objective of **Medicinal Chemistry** that deals with the design, synthesis and biological evaluation of compounds that can be used in medicine for the prevention, treatment or cure of human and animal diseases. Additionally, medicinal chemistry includes the study of existing drugs, of their biological properties and of their structure-activity relationships. As such, the final objective of medicinal chemistry and, therefore, drug design, is to help human kind to overcome the problems associated with illnesses.

Before describing the process of designing a drug, one needs to consider how a drug exerts its biological effect. If we consider oral administration, the drug, goes through many stages once swallowed. First, it arrives at the stomach and intestines where it will be adsorbed in the blood stream. Then, it will be distributed through the blood vessels to reach the organ where the drug has to produce its effect. Here it will interact with a macromolecule known as the target or receptor. As a consequence of this interaction a complex will be formed and this will start a cascade of events that will end in the physical change that we perceive as the effect of the drug.

Drug discovery

In the discovery of a drug we can consider two phases: that of design and synthesis and that of development. The first phase consists of three steps: selection of the objective or

target, identification of a prototype or lead compound and then its optimisation. And, in the development phase we can distinguish between the pre-clinical study, the clinical phases and the post-marketing studies.

The target selection is fundamental in the design and synthesis phase, because this is when the biological process and, if possible, the macromolecules (proteins, enzymes, nucleic acids) involved in such a process are identified. For example, if we want to find a drug to treat depression one of the possible targets will be some macromolecules in the brain, for example the alpha2-adrenergic receptors. Some targets are evident, such as enzymes involved in metabolic processes that need to be 'switch off' by inhibitors, (Patrick: 2005) DNA for anticancer agents to avoid the reproduction of the cancer cells, (Patrick:2005) or the processes involved in the viral replication cycle for anti-AIDS drugs. (De Clerq: 2002). As well, recent advances in the identification of the human genome are allowing the identification of new targets. (Harris: 2000). In a second step we will try to find a prototype drug or a 'lead-compound' which shows a certain activity or affinity towards the chosen target. And then, we will optimise this lead-compound - this means, we will try to improve its activity, to make it more selective towards the target and to diminish its toxicity or unwanted secondary effects.

How can we find a prototype or 'lead compound'? There are two main ways to identify a new 'lead': rational design and high throughput screening. One of the options of rational design is that known as computer assisted drug design or molecular modelling, which consists in trying to match the structural requirements of the ligand (drug) with those of the target by means of computational techniques such as docking (Meng, Shiochet and Kuntz: 1992) and molecular dynamics. (Haile: 1992; Leach: 1996). This methodology can be used when both the 3D structure of drug and target are known. Then, with the help of certain programs, the structures will be represented in the computer and by using different techniques (docking, molecular dynamics) one can make them fit into each other. Even when the 3D structures of target and ligand are not known, there are other computational techniques that allow the molecular modelling, such as the development of a theoretical 'pharmacophore' (minimum structure/chemical groups responsible for the activity of a drug) (Holtje: 2000), or the modelling of a receptor using 'homology modelling'. (Sali and Blundell: 1993).

Another very common way to discover lead compounds, totally opposite to rational design, would be by 'serendipity,' or, the ability of making fortunate discoveries by accident and sagacity. Some examples of this are: Penicillin discovered by Fleming who

when back at his lab after a summer holiday discovered that mould from one culture caused bacteria in its vicinity to undergo lysis. Other known drugs discovered by serendipity are the tranquilizer Chlorpromazine or Viagra which was originally developed as a drug for heart conditions and it was only later that a certain side effects were noticed.

The other main method to identify prototype drugs, as mentioned before, is by high throughput screening. In 1985, to test 10,000 compounds per year and biological activity was considered acceptable. Today, that amount of compounds can be tested in only one week or, when using the most sophisticated techniques, those 10,000 compounds can be tested in even one hour. To perform these high throughput screenings a large amount of compounds are needed and traditional medicinal chemistry by itself cannot generate such amounts. Two sources can yield such large amount of compounds: natural products and combinatorial chemistry.

Natural products have always been an important source of 'lead compounds' for their pharmacological properties. I will only mention some examples that have shown to be rather relevant. Epibatidine, is a significant and potent analgesic that is obtained from the skin of a frog. (Spande *et al*: 1992). This compound is 400 times more potent than morphine but it is toxic to analgesic doses. Famous taxol is an anticancer agent extracted from the pacific yew (Wall and Wani isolated it from the bark of Taxus brevifolia). (Wall and Wani: 1995).

Combinatorial chemistry

Combinatorial chemistry is the simultaneous synthesis of a large number of compounds known as a combinatorial library. A library may consist of collections of single compounds or groups of mixtures of compounds. These compounds may be synthesised on a solid support (where the starting material is bound to a polymer) or in solution. The idea behind this methodology is that when searching for a compound with a certain biological activity, probability is of importance: the more compounds that are screened, then the higher probability of finding a 'lead compound'. The technique of combinatorial chemistry has dramatically changed the way chemists go about searching for lead compounds. The essence of combinatorial chemistry is the generation of large numbers of compounds very quickly. (Nicolau, Hanko and Hartwig: 2002). The traditional methodology in medicinal chemistry involved the preparation, purification and characterization of individual compounds; if the whole process is accelerated then better progress can be made. Not too

much compound is needed since, in the end, when running a first set of biological assays only a few milligrams are required.

Combinatorial chemistry has its origins in the solid-phase synthesis in parallel first developed by Merrifield in 1963 to prepare polypeptides on a polystyrene support. Since then, the organic synthesis in solid phase has been mainly used to synthesise peptides, and it was not until the 80s when it started to be used to prepare new organic compounds searching for new drugs. The method works by attaching the substrate or 'initial building block' to a solid phase (bead). Then, a reactive (second 'building block') will be added, reacting with the substrate and allowing purification by simple filtration of the solution containing by-products from the solid bead with the two 'building blocks' connected. This process for making peptides works very well and is now an automated process (Coffen and Luithle: 2002).

In some cases there are disadvantages to solid-phase library generation, for example, there are not appropriated solid supports for the compounds we are working with, or it is not possible to detach the final compounds from the 'beads'. A newer approach is to use combinatorial synthesis in solution-phase, that is, without attaching the first 'building block' to the solid support.[12] New equipments have been developed allowing for the preparation at the same time of many compounds, or 'parallel synthesis', in a simple and quick manner by only one chemist. These techniques can make use of robotized technology in the same way that the peptide synthesis on solid support seen above. These robots introduce the solvent, the different reactants after a predetermined time, and control the temperature of the whole process. The final objective of their use is to increase productivity and precision. (de Witt: 2000).

A test example of the parallel synthesis in solution phase was performed by workers at GlaxoWellcome. They studied a synthesis known as Hantzsch synthesis using four ketones and five thioureas to prepare twenty thiazoles with potential anti-inflammatory properties. The compounds were prepared using a robot. (www.hull.ac.uk). Through this combinatorial approach, by using 10 by 10 compounds we would be able to obtain 100 compounds but if we use a four component system with 10 different compounds for each system we would be able to produce a library of 10,000 compounds.

Hence, the final objective of combinatorial chemistry is to prepare a large amount of new molecules in a quick and inexpensive way. At present, this methodology is not only a new synthetic approach, but also has become a sophisticated and automated technology. This means that each company can build its own type of libraries. These libraries can be

large and unspecific or small and specialized, automated or semi-automated, on solid support or in solution, and finally the bio-assay can be performed on the mixtures obtained or purify first and then carry out the screening.

Drug development

All this process until the clinical phase is convergent and all the steps are interrelated and aspects such as metabolism or bioavailability are considered from the very beginning of the drug design process. Hence, the time between obtaining a prototype and presenting a good candidate for clinical phase will be reduced. As we mentioned before, the development phase consists in the preclinical studies, then the different clinical phases and finally, when the drug reaches the market it continues to be under control by means of post-marketing vigilance.

Pre-clinical studies: include *in vitro* tests (receptors, cells, isolated tissue) and then *in vivo* tests or animal studies (mice, rat, rabbit, dog). It often requires 4-6 years of clinical testing of animal toxicity studies. Research pharmacologists usually develop these studies. Clinical phases consist in three different steps: Phase I, II, and III. Phase I: the effects of the drug as a function of dosage are established in a small number of healthy volunteers. Phase I trials are done to determine whether humans and animals show significantly different responses to the drug and to establish the probable limits of the clinical dosage range. Much predictable toxicity is detected in this phase. Pharmacokinetic measurements of absorption, half-life, and metabolism are often done in phase I. Such studies are usually performed in research centres by clinical pharmacologists. Phase II: the drug is studied for the first time in patients with the target disease to determine safety and efficacy. A small number of patients (10-150) are studied in great detail. A broader range of toxicities may be detected in this phase. These trials are usually done in special clinical centres. Phase III: the drug is evaluated in much larger number of patients, sometimes thousands. Using information gathered in phases II and I, trials are designed to minimize errors caused by placebo effects. Phase III studies can be difficult to design and execute and are usually expensive, because of the large number of patients involved and the large amounts of data that must be collected and analysed. The investigators are usually specialist in the disease being treated. Certain toxic effects, especially those caused by sensitisation, may first become apparent in this phase III.

Once approval to market a drug has been obtained the post-marketing phase begins. This consists of: monitoring the safety of the new drug under actual conditions of use in

large numbers of patients, final release of drug for general prescription use, a vigilant post-marketing supervision program, and monitoring of adverse reactions reported by pharmacists and doctors. Many important drug-induced effects have an incidence of 1:10,000 or less (therefore, they cannot be detected in Phases I, II or III).

Conclusions

In sum, between the moment that a 'lead compound' is identified and the moment when this compound reaches the public domain, a period of around 20 years can pass. With all this effort in mind, the ethics behind the whole process is evident. For many pharmaceutical companies, drug discovery has become a very lucrative business. Moreover, some unscrupulous governments use legal drugs for non-legal purposes. Nevertheless, for academics and researchers, the most important reason behind the design of new a drug is to benefit human beings by finding new treatments or improving existing treatments to diseases. The challenge remains to find agreement between the different actors involved in the drug discovery process.

Micro reactors for chemical synthesis.

Paul Watts

Abstract

The miniaturisation of chemical reactors offers many fundamental and practical advantages of relevance to the pharmaceutical and fine chemicals industry, which are constantly searching for controllable, information rich, high throughput and environmentally friendly methods of producing products with a high degree of chemical selectivity. This article explores how miniaturisation may revolutionise chemical synthesis.

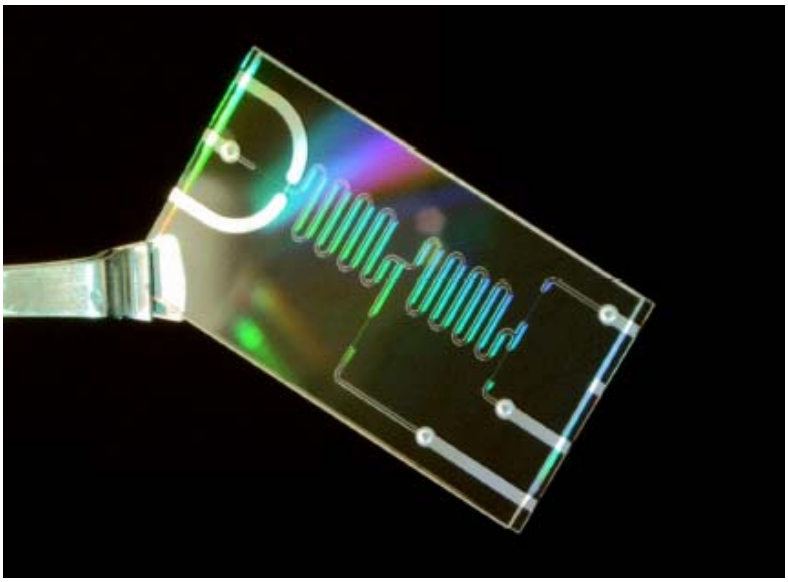
Introduction

Until recently, the greatest research effort in the field of miniaturisation has been in analytical science with the main aim of this research being to develop so called miniaturised Total Analytical System (μ-TAS) (Oosterbroek and van den Berg 2003). Alongside the continuing development of μ-TAS, research is underway to establish the benefits that micro reactors can bring to the field of synthetic organic chemistry. In particular, drug discovery is a very time consuming and expensive process, with one of the slowest steps being the synthesis and purification of potential medicinal candidates. Given that the success of pharmaceutical companies resides largely on their ability to synthesise novel chemical entities and to optimise the production of marketable drugs, the ability to more rapidly synthesise and screen novel chemical entities is clearly desirable and this is starting to be achieved through the use of micro reactor technology.

In their simplest form, micro reactors consist of a network of micron-sized channels (typically 10-300 μm in diameter) etched into a solid substrate (Figure 1). Glass is a popular choice for synthetic applications since it is chemically inert, enables the use of a range of visible light detection methods and fabrication techniques are well established (Ehrfeld, Hessel and Löwe: 2000; McCreedy: 2000, 396). For solution-based chemistry, the channel networks are connected to a series of reservoirs containing chemical reagents to form the complete device with overall dimensions of a few cm. In the micro reactor, reagents can be brought together in a specific sequence, mixed and allowed to react for a specified time in a controlled region of the channel network using electrokinetic or

hydrodynamic pumping (Fletcher et.al.: 2002, 4735). For electrokinetically driven systems, electrodes are located in the appropriate reservoirs (as illustrated in Figure 1) which allow the sequential application of voltages under automated computer control. In comparison, hydrodynamic pumping exploits conventional or micro-scale pumps, notably syringe-type pumps to manoeuvre solutions around the channel network.

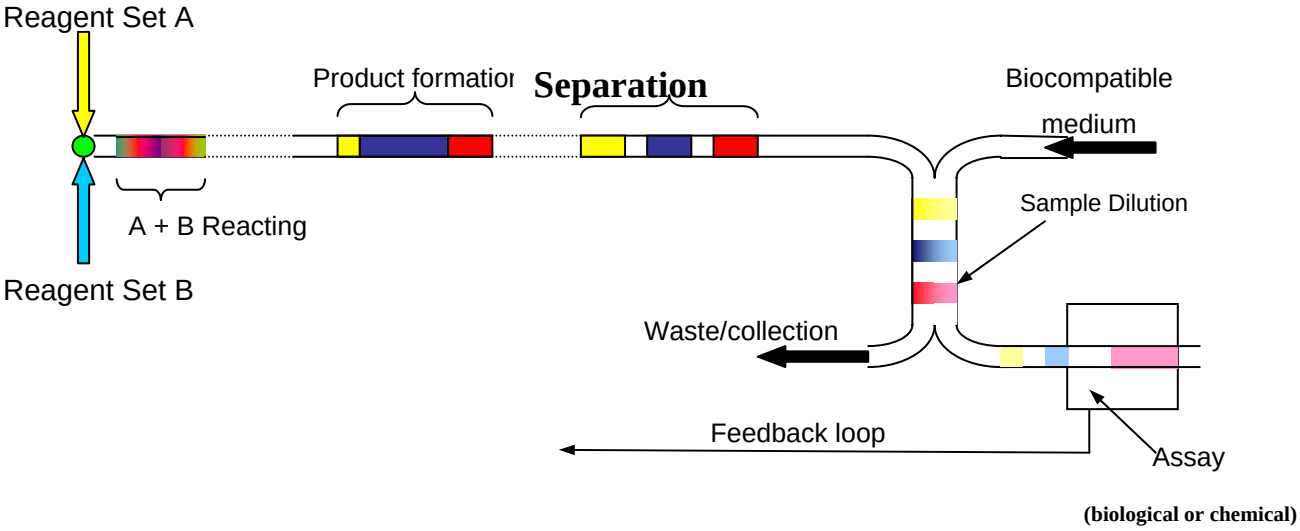
Figure 1. A glass micro reactor containing integrated electrodes, which may be used to generate electroosmotic flow or used for conductivity measurements.



Many chemical reactions have now been demonstrated to show improved reactivity, product yield and selectivity when performed in micro reactors compared to those generated using conventional laboratory practices (Fletcher et al.: 2002, 4735; Jahnisch et al.: 2004, 406; Schwalbe et al.: 2002, 636). Reactions performed in a micro reactor invariably generate comparatively pure products in high yield, when compared to the equivalent bulk reactions, in much shorter times. One of the immediate and obvious applications is therefore in drug and process discovery, where the generation of compounds either with different reagents or under variable conditions is an essential factor. Performing chemical reactions within a microfluidic system also provides the opportunity to perform real time separations. Hence, integration of a micro reactor device *via* a purification/separation step, with one of the many highly sensitive microchannel-based biological assay systems would provide a drug discovery tool. This level of integrated functionality within one device clearly addresses some of industries' requirements for rapid compound production and screening. Apart from the greatly reduced reaction times

demonstrated for the micro reactors, handling times to assay and chemical reagent costs are virtually eliminated with this proposed technology. This is shown schematically in Figure 2.

Figure 2. Integration of a micro reactor with a biological assay system.



In a move to achieve this aim, Garcia-Egido et al. (2002, 170) have recently reported the synthesis of a combinatorial library of pyrazoles within micro reactors operated using hydrodynamic control. A micro reactor was used to react seven 1,3-dicarbonyl compounds with three hydrazine derivatives to produce a library of twenty-one pyrazoles. The automated system consisted of an autosampler to introduce the reagents into the chip, a HPLC pump to move the reagents through the micro reactor and a dilution system to enable a small sample to be diverted to an LC-MS instrument for analysis. The majority of the pyrazoles were obtained in 99% conversion, but clearly the chromatography step allowed the reaction mixtures to be purified to produce analytically pure compounds. The final step would be to couple the output of the chromatography column to a miniaturised bioassay system to enable *in situ* screening to be performed.

Although the above system is excellent in achieving combinatorial synthesis for the desired application, cynics argue that the overall system is hardly miniaturised; the micro reactor itself is tiny but the overall system is still composed of large bench top instrumentation. This is where EOF-based systems are potentially advantageous as external pumps are not necessary and purification could be achieved using on-chip electrophoretic separation rather than using large external instrumentation, such as the HPLC described

above. In a move to develop an EOF based system, Watts et al. (2002, 5427) have demonstrated the first example of multi-step synthesis in a micro reactor where they have used the micro reactors in peptide synthesis. The authors demonstrated that peptide bonds could be prepared in quantitative conversion in minutes, which represented a significant increase in yield compared with the traditional batch syntheses, where only a 50% yield was obtained in 24 h. Although the dipeptide bond forming reactions produced the dipeptide in 100% conversion based on consumption of the acid moiety, the product was still contaminated with residual amine. George et al. (2003, 2886) have reported that the dipeptide may be separated from the reaction mixture by incorporating a capillary electrophoresis channel into the micro reactor chip itself. Hence this methodology enables the synthesis and separation to be efficiently conducted within an integrated micro reactor without the need to have large peripheral equipment attached.

Conclusion

Micro reactor chemistry is currently showing great promise as a novel method on which to build new chemical technology and processes. Reactions performed in a micro reactor invariably generate relatively pure products in high yield, in comparison to the equivalent bulk reactions, in much shorter times and in sufficient quantities to perform full instrumental characterisation. One of the immediate and obvious applications is therefore in combinatorial chemistry and drug discovery, where the generation of compounds either with different reagents or under variable conditions is an essential factor. An interesting twist to the chemistry carried out to date is not just the opportunity to separate reactants and products in real time but also the capability to manufacture and use reagents *in situ*.

References

6th BWC Review Conference (2006) *Draft Final Document*, Geneva, 8 December 2006, available at http://www.opbw.org/rev_cons/6rc/docs/final_dec/BWC+CONF.VI%2BCRP.4-altered+as+ammended.pdf

Aftalion, F (2001) *A History of the International Chemical Industry. From the "Early Days" to 2000*, 2nd Ed., Philadelphia: Chemical Heritage Press.

Anon (2005), 'Public Collections of DNA and RNA Sequence Reach 100 Gigabases', Press Release, National Institutes of Health, 22 August, http://www.nlm.nih.gov/news/press_releases/dna_rna_100_gig.html .

Birren, B, Mancino, V & Shizuya, H (1999), 'Bacterial Artificial Chromosomes', in Birren, B, Green, ED, Klapholz, S Myers, RM, Riethman, H & Roskams, J, *Genome Analysis: A Laboratory Manual. Series 3 Cloning Systems*, New York: Cold Spring Harbor Laboratory Press, pp 241-295.

Boffelli, D, Nobrega, MA & Rubin, EM, 'Comparative Genomics at the Vertebrate Extremes', *Nature Reviews: Genetics*, Vol.5, June 2004, pp 456-465.

Boffelli, D, Weer, CV, Weng, L, Lewis, KD, Shoukry, MI, Pachter, L, Keys, DN & Rubin, EM, 'Intraspecies Sequence Comparisons for Annotating Genomes', *Genome Research*, Vol.14, November 2004, pp 2406-2411.

Boguski, MS & Schuler, GD (1995), 'Establishing a Human Transcript Map', *Nature Genetics*, Vol.10, pp 369-371.

Bremner, SE (2001), 'A Tour of Structural Genomics', *Nature Reviews: Genetics*, Vol.2, pp 801-809.

Butler, D (2004) http://www.nature.com/nature/focus/accessdebate/index.html

The C. elegans Sequencing Consortium (1998), 'Genome Sequence of the Nematode C. elegans: A Platform for Investigating Biology', *Science*, Vol.282, pp 2012-2018.

Chaudary K, and Roos, DS (2005), 'Protozoan genomics for drug discovery', *Nature Biotechnology*, Vol.23, pp 1089-1091.

Clayton, RA, White, O, Ketchum, KA & Venter, JC (1997), 'The First Genome from the Third Domain of Life', *Nature*, Vol.387, pp 459-462.

De Clerq, E (2002), 'New developments in anti-HIV chemotherapy', in *Biochimica et Biophysica Acta*, Vol.258, pp 1587.

Cello J, Paul AV, Wimmer E (2002) 'Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template', *Science*, 297, pp 1016–1018.

Coffen, DL, Luithle, JEA (2002), 'Introduction to Combinatorial Chemistry' in KC Nicolau, R Hanko, W Hartwig (eds.) *Handbook of Combinatorial Chemistry*, Vol. 1, pp 10, Weinheim: Wiley-VCH.

Cooke-Degan, R et.al. (2000) World Survey of Funding for Genomics Research. Final Report to the Global Forum for Health Research and the World Health Organization, September, available at http://www.stanford.edu/class/siw198q/websites/genomics/WorldGenomics$SurveyFinalRpt25Sep00.doc

Cornell Genomics Initiative (2001), 'Enabling Group. Genomic Technologies', available at http://www.genomics.cornell.edu/focus_areas/technology/

Cox, DR, Burmeister, M, Price, ER, Kim, S & Myers, RM (1990), 'Radiation hybrid mapping: a somatic cell genetic method for constructing high-resolution maps of mammalian chromosomes', *Science*, Vol.250, pp 245-50.

Dando, M (1999) 'The Impact of the Development of Modern Biology and Medicine on the Evolution of Offensive Biological Warfare Programs in the Twentieth Century', in *Defense Analysis*, Vol.15, No.1, 1999, pp 43-69.

Davis, SS (1997) 'Biomedical applications of nanotechnology – implications for drug targeting and gene therapy', in *Trends in Biotechnology*, Vol.15, June, pp 217-224.

Drexler, KE (1986) *Engines of Creation: The Coming Era of Nanotechnology*, Garden City, N.Y.: Anchor Books.

Duncan R (2005), *NanoToday*, August, 16-17.

Ehrfeld W, Hessel V, Löwe H (2000) Microreactors: New Technology for Modern Chemistry. Weinheim: Wiley-VCH

EMBL Data Library of the European Molecular Biology Laboratory in Germany, GenBank of the National Center for Biotechnology Information in the USA and DNA Data Bank of Japan. http://europa.eu.int/comm/health/ph_risk/committees/04_scenihr/04_scenihr_en.htm

Fellenberg, M. (2003) 'Developing integrative bioinformatics systems', *Biosilicio*, Vol.1, No.5, November, pp.177-183.

Feynman, R.P. (1960) 'There is Plenty of Room at the Bottom', *Engineering and Science*, Vol.23, pp.22-36, available online at http://www.zyvex.com/nanotech/feynman.html

Fletcher PDI, Haswell SJ, Pombo-Villar E, Warrington BH, Watts P, Wong SYF, Zhang X (2002) 'Micro reactors: principles and applications in organic synthesis', *Tetrahedron* 58: pp 4735

Freitas, R.A., *Nanomedicine*, 2005, **1**, pp 2-9

Garcia-Egido E, Wong SYF, Warrington BH (2002) 'Synthesis of a three-member array of cycloadducts in a glass microchip under pressure driven flow', *Lab on a Chip* 2, p 170

George V, Watts P, Haswell SJ, Pombo-Villar E (2003)' On chip separation of peptides prepared within a micro reactor', *Chemical Comm*., Vol. 2886

Global Grid Forum, 2005, http://www.gridforum.org/

Gregory, SG, Howell, GR & Bentley, DR (1997), 'Genome Mapping by Fluorescent Fingerprinting', *Genome Research*, Vol.7, Issue 12, December, pp 1162-1168.

Grid.org (2005), http://www.grid.org/home.htm

Gross, M (1999) *Travels to the Nanoworld*, New York : Plenum Trade.

Haile, JM (1992) *Molecular Dynamics simulations*, Wiley Interscience.

Hanash, S (2003) 'Disease Proteomics', *Nature*, Vol.422, March, p 230.

Harris, T (2000) 'Genetics, Genomics and Drug Discovery', *Medicinal Research Reviews*, 203, *20*.

Holland, J and Mitchel, T (1999) 'Chemists Harness IT to Organize Data and Optimize Leads', in *R&D Magazine*, Vol.41, No.10, pp.23f.

Holtje, H-D (2000) 'Pharmacophore identification and receptor mapping' in CG Wermuth (ed.) *The Practice of Medicinal Chemistry*, Oxford: Academic Press.

Huff, JL Lynch, MP, Nettikadan, S, Johnson, JC, Vengasandra, S & Henderson, E (2004), 'Label-Free Protein and Pathogen Detection Using the Atomic Force Microscope', *Journal of Biomolecular Screening,* Vol. 9, September, pp 491–497.

Hughes, TR, Marton, MJ, Jones, AR, Roberts, CJ, Stoughton, R, Armour, CD, Bennett, HA, Coffey, E, Dai, H, He, YD, Kidd, MJ, King, AM, Meyer, MR, Slade, D, Lum, PY, Stepaniants, SB, Shoemaker, DD, Gachotte, D, Chakraburtty, K, Simon, J, Bard, M & Friend, SH (2000) 'Functional discovery via a compendium of expression profiles', *Cell,* Vol.102, July, pp 109–126.

www.hull.ac.uk/php/chsanb/DrugDisc/IntroDrugDiscovery.pdf

International Human Genome Sequencing Consortium (2001), 'Initial Sequencing and Analysis of the Human Genome', *Nature*, Vol.409, February, pp 860-921.

Jahnisch K, Hessel V, Lowe H, Baerns M (2004) 'Chemistry in Microstructured Reactors' *Angewandte Chemie International Edition* Vol.43, p 406

Kelle, A, Nixdorff, K, Dando, M (2006) *Controlling Biochemical Weapons. Adapting Multilateral Arms Control for the 21st Century*, Basingstoke: Palgrave Macmillan.

Kitano, H (2004a) 'Cancer as a robust system: Implications for anticancer therapy', *Nature Reviews – Cancer*, 4, pp 227-235.

Kitano, H (2004b) 'Biological robustness', *Nature Reviews – Genetics*, 5, pp 826-837.

LaCount, DJ, *et al.* 'A protein interaction network of the malaria parasite Plasmodium falciparum', *Nature*, 438, pp 103-112.

Lander, ES *et al*. (2001) 'Initial Sequencing and Analysis of the Human Genome', *Nature*, Vol. 409, February, pp 860-921.

Leach, AR (1996) *Molecular Modelling: Principles and Applications*, ORT: Prentice Hall, 2nd Ed.

Lockhart, DJ & Winzeler, EA (2000) 'Genomics, Gene Expression and DNA Arrays', *Nature*, Vol.405, June, pp 827-836.

Marlow, JR (2004) *NANO*, New York: Forge.

Marra, MA, Kucaba, TA, Dietrich, NL, Green, ED, Brownstein, B, Wilson, RK, McDonald, KM, Hillier, LW, McPhearson, JD & Waterson, RH (1997) 'High Throughput Fingerprint Analysis of Large-Insert Clones', *Genome Research*, Vol.7 Issue 11, November, pp 1072-1084.

Martinetz, D (1995) *Vom Giftpfeil zum Chemiewaffenverbot – Zur Geschichte der chemischen Kampfmittel*, Frankfurt/Main: Verlag Harri Deutsch.

McCreedy T (2000) Trends in Analytical Chemistry, 19; 396

Meng, EC Shiochet, BK Kuntz, ID (1992) 'Automated docking with grid-based energy evaluation", *Journal of Computational Chemistry,* 505, *13*.

Messing, J & Llaca, V (1998) 'Importance of Anchor Genomes for any Plant Genome Project', *Proceedings of the National Academies of Science*, Vol.95, March, pp 2017-2020.

Millett, P (2006) 'Scientia Corrupta: The Life Sciences and Biological Weapons', in Pascal, JP (Ed), *BioWeapons Report 2005*, Geneva: BioWeapons Prevention Project, forthcoming.

Moghimi SM, Hunter CH, Murray JC (2005), 'Nanomedicine: current status and future prospects', *FASEB Journal*, Vol.19, No.3, pp 311-330.

The National Academies (2004) *Biotechnology Research in the Age of Terrorism,* Washington D.C.: The National Academies Press.

The National Academies (2005) *An International Perspective on Advancing technologies and Strategies for Managing Dual-Use Risks*, Washington, DC: The National Academies Press.

The National Academies (2006) *Globalization, Biosecurity, and the Future of the Life Sciences*, Washington, DC: The National Academies Press.

Nicolau, KC Hanko, R Hartwig, W (2002) 'Combinatorial Chemistry in perspective' in *Handbook of Combinatorial Chemistry*, Ed.: KC Nicolau, R Hanko, W Hartwig, Vol. 1, pp 3, Weinheim: Wiley-VCH.

NSTC (2000) *Definition of nanotechnology*, June 12, available at http://www.becon.nih.gov/nstc_def_nano.htm, accessed 12 January 2006

Oberdörster, G, Oberdörster, E and Oberdörster, J (2005) 'Nanotoxicology: An Emerging Discipline Evolving from Studies in Ultrafine Particles', *Environmental Health Perspectives*, Vol.113, No.7, pp 823-839.

Oosterbroek, RE, van den Berg, A (2003) (eds) *Lab-on-a-Chip: Miniaturised Systems for (Bio)Chemical Analysis and Synthesis*. Amsterdam: Elsevier.

OPCW (2006) *Opening Statement by the Director General to the Conference of the States Parties at its Eleventh Session*, The Hague: OPCW, Document C-11/DG.9, dated 5 December 2006

OSI, (2005) Open Source Initiative, http://www.opensource.org/

Patrick, GL (2005) 'Proteins as drug targets: enzymes' in *An Introduction to Medicinal Chemistry*, Oxford: Oxford University Press.

Patrick, GL (2005) 'Nucleic acids as drug targets' in *An Introduction to Medicinal Chemistry*, Oxford: Oxford University Press.

Patterson, SD and Aebersold, RH (2003) 'Proteomics: The First Decade and Beyond', *Nature Genetics Supplement*, Vol.33, March, pp 311-323.

Pearson, A (2006) 'Modest Progress at the Sixth Review Conference', available at http://www.bwc06.org/ entry dated 11 December 2006.

Preparatory Committee for the 6th BWC Review Conference (2006) *Report of the Preparatory Committee*, Geneva: United Nations, 3 May, document BWC/CONF.VI/PC/2, accessed at http://daccessdds.un.org/doc/UNDOC/GEN/G06/611/97/PDF/G0661197.pdf?OpenElement.

RAND (2001) *The Global Technology Revolution. Bio/Nano/Materials Trends and Their Synergies with Information Technology by 2015*, Prepared for the National Intelligence Council, Santa Monica: RAND.

Ritchie, T (2001) 'Chemoinformatics; manipulating chemical information to facilitate decision-making in drug discovery', in *Drug Discovery Today*, Vol.6, No.16, pp 813-814.

Robinson, JP (1998) 'The Negotiations on the Chemical Weapons Convention: a historical overview', in, M. Bothe/N. Ronzitti/A. Rosas (eds.), *The New Chemical Weapons Convention – Implementation and Prospects*, The Hague: Kluwer Law International, pp 17-36.

Royal Society (2004) *Nanoscience and nanotechnologies: opportunities and uncertainties*, London, 29 July, available online at http://www.nanotec.org.uk/finalReport.htm

Sahoo, SK & Labhasetwar, V (2003) 'Nanotech approaches to drug delivery and imaging', in *Drug Discovery Today*, Vol.8, No.24, pp 1112-20.

Sali, A & Blundell, TL (1993) 'Comparative protein modelling by satisfaction of spatial restrains', *Journal of Molecular Biology*, 779, *234*.

Schwalbe T, Autze V & Wille G (2002) 'Chemical synthesis in microreactors', *Chimia* 56: 636.

Searls, DB (2003) 'using bioinformatics in gene and drug discovery', Drug Discovery Today, Vol.5, No.4, pp 135-143.

Secretariat of the 6th BWC Review Conference (2006) *Background Document on New Scientific and technological Developments Relevant to the Convention*, Geneva: United Nations, document BWC/CONF.VI/INF.4, 28 September, accessed at http://www.unog.ch/80256EE600585943/(httpPages)/3496CA1347FBF664C125718600364331?OpenDocument.

SIPRI (1971), *The Rise of CB Weapons*, Vol.I in the series *The Problem of Chemical and Biological Warfare*, Stockholm: Almqvist & Wiksell.

Spande, TF Garraffo, HM Edwards, MW Yeh, HJC Pannell, L Daly, JW (1992) 'A novel (chloropyridyl)azabicycloheptane with potent analgesic activity from an Ecuadorian poison frog', *Journal of the American Chemical Society*, 3475, *114*.

Speicher, D (2002) 'Proteomics: An Infinite Problem with an Infinite Potential', *The Scientist*, Vol.16 No.8, April, pp 12.

Steinmetz, LM & Davis, RW (2004) 'Maximizing the Potential of Functional Genomics', *Nature Reviews: Genetics*, Vol.5, March, p 191.

Tucker, J (1999) 'Biological Weapons in the Former Soviet Union: An Interview with Dr. Kenneth Alibek', in *The Nonproliferation Review*, Vol.6, No.3, pp 1-10.

United Nations General Assembly (1971), 'Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction', New York, USA, A/2826(XXVI) Annex, 16 December.

Van Eerdewegh, P et al. (2002) Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness', *Nature*, Vol.418, pp 426–430.

Venter, JC *et al* (2001) 'The Sequence of the Human Genome', *Science*,Vol.291, February, pp 1304-1351.

Vogt, G (2002) 'Multi-axis robots bring automation to life sciences', in *Industrial Robot*: *An International Journal*, Vol.29, No.1, pp 49-52.

Wall, ME, Wani, MC (1995) 'Paclitaxel- from discovery to clinic', *ACS Symposium Series*, 18, *583*.

Watts P, Wiles C, Haswell SJ, Pombo-Villar E (2002) 'Solution phase synthesis of β-peptides using micro reactors', *Tetrahedron* 58, 5427

Wheelis, M (1999) 'Biological Sabotage in World War I' in Geissler E and van Courtland Moon, JE (eds.) *Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945*, SIPRI Chemical & Biological Warfare Studies, no.18, Oxford: Oxford University Press, pp 35-62.

Wheelis, M (2002) 'Biotechnology and Biochemical Weapons', *The Nonproliferation Review*, Vol.9, No.1, pp 48-53.

White, KP (2001) 'Functional Genomics and the Study of Development, Variation and Evolution', *Nature Reviews: Genetics*, Vol.2, July, pp 528-537.

D. Williams (2005) *Medical Device Technology*, November, pp 6-8.

Wilson, M et al (2002) *Nanotechnology, Basic science and emerging technologies*, Boca Raton, FL: Chapman and Hall/CRC.

Wilson, K & Mardis, ER (1999) 'Fluorescence-based DNA Sequencing', in Birren, B, Green, ED, Klapholz, S, Myers, RM, Riethman, H & Roskams, J (1999) *Genome Analysis: A Laboratory Manual. Series 1 Analyzing DNA*, New York: Cold Spring Harbor Laboratory Press, pp 301-395.

de Witt, SH (2000) 'Combinatorial Libraries and High-Throughput Synthesis' in *The Practice of Medicinal Chemistry*, Ed. CG Wermuth, London: Academic Press.

Wood, A & Scott, A (2000) 'Combinatorial Chemistry Picks Up Speed' in *Chemical Week*, Vol.162, No.30, 9 August, pp 39-42.

Worlock, K, 2004, The pros and cons of Open Access, http://www.nature.com/nature/focus/accessdebate/

Workshop Participants

Adeniji Adesina, Innovata plc

Jürgen Altmann, Experimentelle Physik III, University of Dortmund, Germany

Francisco Azuaje, Reader in Artificial Intelligence, University of Ulster at Jordanstown

J. Anthony (Tony) Byrne, Head of Photocatalysis Research Group, Nanotechnology Research Institute of Northern Ireland, University of Ulster at Jordanstown

Malcolm R. Dando, Professor of International Security, Department of Peace Studies, University of Bradford

Gillian Keane, Student Pugwash Group, Queen's University Belfast

Alexander Kelle, Lecturer in International Politics, School of Politics, International Studies and Philosophy, Queen's University Belfast

Piers Millett, Member of the Meetings Secretariat of the Biological Weapons Convention, 2001 – 2005

Lorna Miller, dstl Porton Down

Kathryn Nixdorff, Professor of Microbiology and Genetics, Technical University Darmstadt, Germany

Julian P. Perry Robinson, Professor, Science Policy Research Unit, University of Sussex, Co-director Harvard-Sussex Program on CBW

Isabel Rozas, Lecturer in Medicinal Chemistry, School of Chemistry, Trinity College Dublin, Ireland

Katie Smallwood, Doctoral Candidate, HSP, University of Sussex

Ralf Trapp, Consultant, formerly: Senior Planning Officer, Organisation for the Prohibition of Chemical Weapons, The Hague, The Netherlands

Paula Vanninen, Director, VERIFIN, Finland

Paul Watts, Lecturer, Department of Chemistry, University of Hull

Jim Whitman, Lecturer, Department of Peace Studies, University of Bradford