Brave small world

Biotechnology and nanotechnology may give rise to a completely new industry

Nanotechnology resurfaces regularly in the popular and scientific press. But despite the pretty pictures of 'nano submarines' patrolling the body for tumour cells in popular magazines and miniature cogs on the cover of *Nature*, there are few applications of nanotechnology that have actually made it to the market place. So

meter scale, the differences between disciplines disappear.' Gaub's group studies the biophysics of single molecules, in particular the folding processes of protein molecules. By attaching the tip of a piezoelectric cantilever to the end of a membrane protein and pulling, the protein can be observed unravelling in the reverse

this research that he has started his own company to commercialise his invention (<http://www.nanotype.de>).

Germany, perhaps because of its historical strength in chemistry and physics, and present day reliance on these industries, is taking a very keen interest in nanotechnology. A growing number of institutes specialising in physics and chemistry are exploring the realms of nano research. 'Nanotechnology and biotechnology are two fields of research with huge potential for development at their interface,' writes the bmb+f (Federal Ministry for Education and Research) on its nanotechnology website (<http://www.nanonet.de/>). The German government strongly supports and funds basic research in nanotechnology, in particular collaborations between the fields of nano research and biotechnology.

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far, dirt-repelling surface coatings and paint additives are the only commercial applications to result from nano research. Miniature machines and submarines may be some way off, but in the biomedical sector a fledgling industry is emerging that exploits nanotechniques for diagnosis and drug delivery.

It was the physicist and Nobel laureate Richard Feynman who saw the day when it would be possible to build miniature devices bottom-up, starting with single atoms or molecules. This differs from conventional miniaturisation techniques, which are top-down. They start with larger substrates, and make it smaller by taking matter away—the miniature cogs in *Nature* are such an example.

Nanoscience today is often defined as the study of systems of the order of 1 to 100 nm in size, although larger structures, for example those made by etching silicon, have been referred to as 'nano' as well. On one thing all agree: nanotechnology and its applications are proving a middle ground for the meeting of intellects from a wide diversity of scientific backgrounds; the life sciences, physics, chemistry, engineering, electronics and chemistry to name some.

As Hermann Gaub, Chair of Applied Physics at the Ludwig Maximilian University in Munich, remarked, 'at the nano-

order of the folding process during membrane insertion. Gaub and co-workers at the Max Planck Institute for Biochemistry in Martinsried, Germany have demonstrated this principle with the bacterial light-harvesting protein rhodopsin (Oesterhelt *et al.*, 2000).

An increasing number of structural biologists are already using this method because it can give very controlled access to the functional parameters of proteins, and is indispensable for measuring the mechanical properties of structural proteins such as the muscle protein titin. However, according to Gaub, the technique could easily be expanded to investigate protein-protein interactions. As he pointed out, binding partners that interact over equivalent areas may have very different forces of interaction. Measuring the force needed to separate two molecules using nano-cantilevers can, therefore, distinguish between specific and non-specific binding modes.

Gaub thinks that the first exploitation of this technology will be in the pharmaceutical sector, where the strength of binding of drug molecules to their intended biological target could be assayed. As he noted, the technology will lead to more precise, better and faster assays when applied to genetic diagnostics and genotyping. So convinced is he of the commercial value of

As Gaub mused, 'at the nanometer scale, the differences between disciplines disappear.'

Dietmar Wechsler of the bmb+f, rates the prospects for nano biotechnology in Germany as good, though not as good as those in the USA or UK, where the financing of this research is stronger. The bmb+f recently decided to devote significantly more money to biotechnology in general over the next few years and, in December 2000, it announced a 7.3% increase in expenditure on biotechnology to Euro 110 million. It is also supporting joint projects in nano biotechnology with the BEO (Projekträger Biologie, Energie, Umwelt des BMBF und des BMWi) to the tune of Euro 20–25 million over the next three years. 'The nano biotechnology industry will grow in Europe despite competition from the USA because of the diversity of fields into which it is branching,' said Wechsler. There is plenty of

scope for new patents, and in his assessment, applied research in nano biotechnology will make an impact in Germany, probably initially in gene therapy, biochips and diagnostics. Molecular electronics based on biological materials, for example optically activated transistors, might develop in the more distant future; a future that Wechsler certainly sees as promising for young scientists wishing to work in nanotechnology in Europe.

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In the USA, small things are also looking up. President Clinton pledged \$500 million to nanotechnology research last year, a characteristically bold move that reaffirms America's great faith in the ability of basic research to generate economically exploitable results. Indeed, John Zhang, assistant professor at the Georgia Institute of Technology in Atlanta, GA, noted that his work on magnetic nanoparticles has already aroused interest in the biotechnology industry. Because his nanomagnets can be guided by external electric fields, they could be used to deliver drugs to disease sites in the body (Figure 1).

The potential benefits are huge. If a drug can be localised where it is needed, some of the most hindering aspects of drug design disappear: systemic drug toxicity will cease to be a problem. Doctors will not need to flood the body with excess drug to ensure that enough reaches the target. Nor will the pharmaceutical industry have to spend considerable resources on modifying the active moiety of a drug to be efficiently taken up in the gut, or to withstand so-called 'first pass' degradation in the liver. Drugs attached to magnetic nanoparticles need never see the liver since the particles carrying them would be injected into the bloodstream and localised immediately. This also promises much reduced side-effects.

Having been steered magnetically to the correct general area, biological recognition of a ligand by a cell surface receptor completes the process of targeting.

1. External magnetic field - guiding the magnetic drug carriers near the tumour.

2. Surface antigen recognition - attaching the carriers to the tumour for drug release on site.

electron microscopy image of magnetic nanoparticles

Fig. 1. Drug delivery using magnetic nanoparticles.

Zhang noted that 'you have to make the particles friendly to the immune system', to avoid inactivation. Also, they must be large enough to remain in the body for the desired time, and must not clump together in the patient's blood. At the same time, they must be magnetically strong enough to be localised by weak external fields.

Though the technology is certainly state of the art, the idea itself is '40 or 50 years old,' according to Zhang. But until

recently, magnetic nanoparticles did not have sufficiently strong magnetic properties, and could only be moved a few millimetres. 'What you need is a super-paramagnetic particle,' said Zhang, 'and this is a big problem'. Evidently not insurmountable, because Zhang's group have developed a material that has a super-paramagnetic transition temperature below that of the body. Now it is a case of collaborating with clinicians to initiate animal trials of the magnetic nanoparticles. Georgia Tech has recently founded a collaboration with Emory University under the title 'Biomedical Engineering'. The potential for drug delivery, especially against cancer, is enormous. 'Just imagine what could be achieved if a nanoparticle could enter a cell and act as a modulator of signal transduction,' mused Zhang.

Picking up molecules with nanotweezers is unlikely to move mountains in practical terms, but for structural and functional analysis nanotubes can have important uses

Professor Charles Lieber from Harvard University in Cambridge, MA, is working on an even smaller scale. He is famous for his nanotweezers made from two carbon nanotubes, which can be brought together by applying a voltage across them. Picking up molecules with nanotweezers is unlikely to move mountains in practical terms, but as probes for structural and functional analysis nanotubes can have important uses. In conjunction with atomic force microscopy they can be used to map the shape of a molecule at a resolution of 5–10 Å, and are a useful analytical tool in haplotyping (Woolley *et al.*, 2000). Essentially the DNA is tagged with a molecule that creates a recognisable feature in a sequence-specific manner. The carbon nanotube then probes the shape, and hence sequence, of the DNA. However, 'whether base-by-base sequencing is practically going to happen is another question,' conceded Lieber soberly.

Another potential use for carbon nanotubes is in the gross determination of protein complex orientation. Though attaining a lower resolution than X-ray crystallography, a nanoprobe could still provide useful information on the orienta-

tion of two or more proteins in a complex. One of Lieber's intended objects of study is the transcriptional regulation complex dubbed the 'enhanceosome', which consists of eight proteins.

The most promising line of research in terms of large-scale application is that of semiconductor nanowires. Having a similar diameter to carbon nanotubes, semiconductor nanowires differ importantly in having electrical properties that can be readily modulated and controlled. 'What happens at the surface really affects the whole material,' said Lieber. In effect, the binding of a single molecule to a nanowire can be detected by a change in conductance. And with these few words, an enormous valley of opportunity opens in which silicon chip microarray technology is seen to give way to much smaller grids of semiconductor nanotubes. As Lieber explained, using such a grid—the junctions of which operate as read-in and read-out nodes—one could 'exploit all the chemistry of chip technology without the need for fluorophore labelling'. This is

because the wires are so sensitive that amplification of the original signal by light emitting chemical reactions (as is the case with conventional microarrays) would be unnecessary. Lieber sees a future where direct electrical measurements of binding essentially replace labels and optical scanning. In a proof-of-principle experiment, he has already shown that such functional nanogrids are feasible (Duan *et al.*, 2001). Companies are already showing interest in developing such technology into commercial screening kits, according to Lieber.

As in all branches of nano research, the potential seems immense, but massive interdisciplinary research is still needed to make the ideas applicable and broaden their scope. Information emerging from the human genome project will turn up countless targets for nano research. 'Some things will emerge that are commercially useful,' noted Lieber; biosensors, for instance, could make an impact fairly soon. Despite the exaggerated claims of the past, venture capitalists in the USA are

clearly showing interest in nanotechnology. 'My hope is that there are some very tangible effects that the lay person will see,' Lieber concluded. These may still be a few years off, but this time the claims are not pure 'nanohype'.

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What is patent-worthy?

Due to differing legal standards for patentability, applications for gene patents face different outcomes in various countries

The United States Patent and Trademark Office granted four gene patents to Sagami Chemical Research Institute in Sagami City for human cDNAs related to liver function and cell division, although the exact function and mechanism of the coded proteins were unknown. Earlier last year, the Japanese Patent Office had rejected these applications, demonstrating, according to the Japanese business newspaper *Nikkei* (January 8, 2000), that the USPTO has a more relaxed stance in granting gene patents.

Indeed, it is not unusual that a patent for the same invention is granted in one country and denied in another, because the legal tests for patentability are not the same in every jurisdiction. Although inventors can file under the Patent Cooperation Treaty with most patent offices in

the world to receive protection of their invention, the individual outcome still depends on the patent office in each country. Particularly when it comes to granting patents for expressed sequence tags (ESTs)—when found by sequencing or by mere homology searches in databases—the USPTO has been shown recently to decide differently from its European and Japanese counterparts.

However, a comparative statement, such as the one the Japanese newspaper made, may be too simplistic. 'Among patent practitioners, the laws of the US, Europe and Japan are considered to be the most developed in the biotech field,' S.Peter Ludwig, senior member of Darrby & Darby PC, an intellectual property law firm in New York, said, 'but they are certainly not "equivalent"'. In some respects

Japanese law on patentability is more lenient than US law, in others, more stringent.'

International differences in intellectual property laws and their consequences for the biotech industry were among the topics of the BioJapan 2000 symposium in September last year in Tokyo. Patent attorneys and patent officers from Japan, Australia, the UK, Germany and the United States expounded about international strategies for biotech patents. Among the speakers, Janis K. Fraser of Fish & Richardson PC, an intellectual property law firm in Boston, MA, compared the criteria for patentability of genes that the US, European and Japanese patent offices apply. All three accept patent applications for chromosomal genes, cloned cDNA and virtual cDNA discovered by data mining. According to