Peptides as Drugs

Peptide research has seen progressive growth over the past few decades, in particular with respect to 'peptide therapeutics'. Many companies specialising in their manufacture, along with companies developing peptide-based products ranging from new drug candidates to medical diagnostic devices, through to cosmetics and food technologies, have come to the forefront of pharmaceutical acquisitions and venture capital groups.

What are Peptides?

Peptides, typically classed as molecules containing between two and fifty amino acids bonded together, and proteins (larger peptide molecules containing over fifty amino acids, 'polypeptides') have long been regarded as crucial to offering solutions to mounting and increasingly difficult world health issues, and the possibility of patient-specific therapy.

Peptides and proteins are found throughout biology, and possess a range of physiological and cellular functions. Their structure is often complex, capable of presenting in many different conformations, dependant upon their environment. It is perhaps in part due to the complexity of the tertiary and quaternary structures that the peptide-drug market stalled somewhat between the development of insulin in 1959 and the last decade. Other factors which have caused problems with development are the short half-life of peptide molecules due to their rapid metabolism in body, and also the delivery to specific organs.

Acceptance over the last decade by the science community, that peptide/protein drugs are viable options for therapy, has driven research organisations into screening libraries of peptides, looking for bio-interactions and potential lead candidates. The overall process of drug discovery can be summarised broadly as three separate components: the initial stage of drug discovery, investigating protein behaviour and characteristics; the second stage of discovery, identifying potential candidates that can bind to the protein and adjust its behaviour; and the third stage, testing of a lead candidate's effect on the native protein. For each of these stages, the use of peptide molecules assists research, and these reagents are seen as a key influence in future drug discovery.

With a clearer understanding of aspects of structural biology and drug metabolism pharmacokinetics, research into peptide/protein-based drugs has started to flourish, with the ability to deliver the drugs to specific sites, and the drugs offering high potency, and importantly, low toxicity. These key factors differentiate the peptide/protein therapeutics from more traditional 'small molecule' drugs, and leads science to ask "Why is this so?"

At the molecular level, peptides influence the majority of physiological processes. They act by binding to specific cell surface receptors, and modulating the protein's activity. The specificity obtained from using peptide therapeutics can be attributed to the complex structures of both the labile peptide, which may adopt a specific conformation allowing for interaction with only specific corresponding sites, and to the quaternary structure formed upon interaction with the protein. The generally lower toxicity of peptides can be attributed to both their lower instance of interaction with other molecules not of interest (which may be other drug entities or other proteins in the body), and also to their ease of metabolism into their component amino acid residues.

Does the Indication Mean that Peptide Drugs have a Long and Secure Future?

There has been a large growth over recent years in peptide drugs and drug candidates. In 2010, there were a total of sixty peptide drugs that had, at that point, been approved in the USA by the Food and Drug Administration (FDA). There were also 140 peptide drugs in clinical trials, and over 500 in pre-clinical development. With the majority of clinical trials targeting oncology, cardiovascular, metabolic and infectious diseases, there is a large opportunity to explore peptide therapeutics for other medical disorders.

Of particular interest are the peptides targeting metabolic disorders, with the estimation of obesity being over 100 million people globally and increasing. This area of research has seen some significant breakthroughs, with the discovery of leptin, an adipose tissue peptide hormone intensifying studies. Leptin has been shown to act by decreasing appetite and increasing metabolic rate in studies in animals. Studies into this, and analogous peptides will continue to expand, and it is hoped that it could lead not only to treatments for obesity, but also to an antagonist of the mechanism, which could be employed in the treatment of anorexia. As obesity levels increase and concurrently generate more dependence upon treatment for diabetes, biomedical research into insulin-like peptides will continue.

Insulin was the first peptide to be administered therapeutically, and has been used for over half a century in the treatment of diabetes. Despite research not offering suitable small molecule alternatives to insulin, there have been developments into novel analogues, such as lispro insulin. This is shorter-acting, allowing for a more convenient injection just prior to a meal compared with insulins that are injected thirty minutes prior to a meal. There have also been developments into non-injectable forms of insulin.

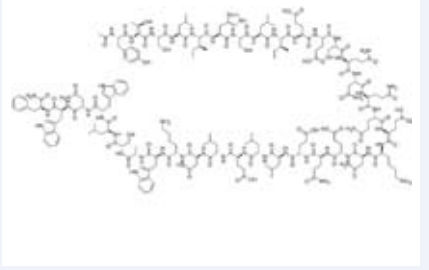
Another peptide of interest is glucagon-like peptide-1 (GLP-1), which shows insulin-releasing properties and also suppresses glucagons levels, whilst

delaying gastric emptying. The action of GLP-1 is glucose-dependant and as such it is more potent following a meal.

Peptides drugs having multiple medical benefits have also reached the market, such as octreotide. This long-acting stable analogue of somatostatin has a number of therapeutic indications, having been used effectively in the treatments of acute pancreatitis, upper gastrointestinal bleeding, gastro-enteropancreatic endocrine tumours and acromegaly amongst others. Further developments of this analogue have led to lanreotide and Sandostatin LAR, having longer-acting lifetimes and thus requiring less frequent injections.

In 2003, Roche marketed the peptide enfuvirtide as Fuzeon for application in combination therapy towards the treatment of HIV-1 infection. The peptide, an HIV fusion inhibitor shown below, demonstrates the complexity of a peptide drug's chemical structure as compared to more tradition small molecule drugs. Enfuvirtide was discovered at Duke University, by a small pharmaceutical company called Trimeris, formed by researchers at the University. After three years of development, Trimeris partnered with Hoffman-La Roche in 1999 to complete the development of the drug, and achieved FDA approval in March 2003 as the first HIV fusion inhibitor. It is estimated that the use of Fuzeon in therapy costs around $25,000 per person per year in the US.

Enfuvirtide, marketed as Fuzeon, has the amino acid residue sequence: Ac-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-NH2



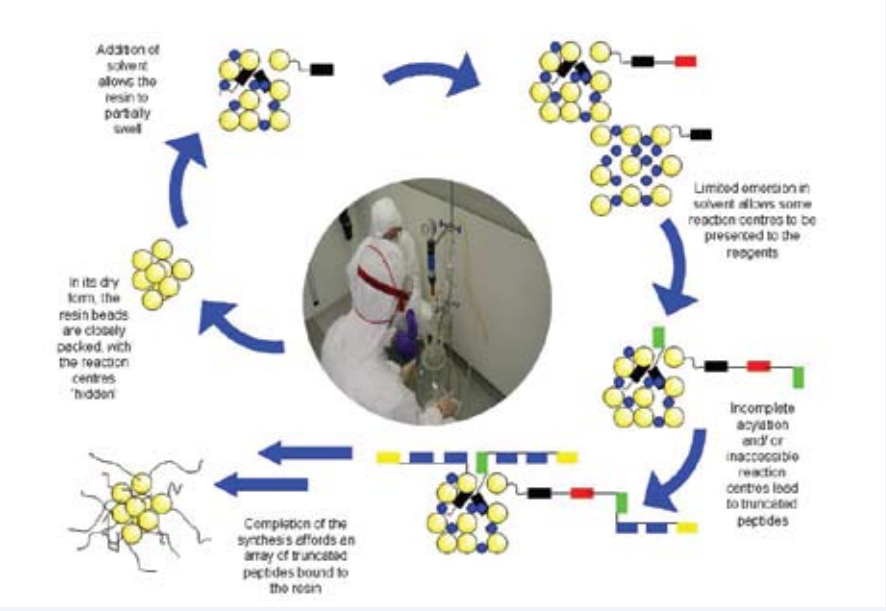
An area of biomedical research which has drawn increasing research for many years is that of antibiotic peptides. There have been two antibiotic peptides polymyxin B and polymyxin E (also known as colistin) that are already licensed for use. Colistin has been employed via injection, orally and by aerosol, and has been used to treat Pseudomona aeruginosa in cystic fibrosis patients. Polymyxin B has been used for the treatment of eye and ear infections and is employed topically. Research has identified that further classes of antibiotic peptides exist including magainins, defensins and cecropins amongst others. With the increasing ineffectiveness of traditional antibiotics, the research into peptide-based antibiotics will become essential for world health organisations.

Increasingly, across the broad range of therapeutic areas the structures of these target compounds have become larger (more amino acids conjugated in the primary structure) and more complex. In order for these to be viable options for the healthcare industry, the technology to manufacture these compounds has had to develop considerably. Sustaining accessibility to the increasingly complex peptide species, the larger pharmaceutical companies, biotech SMEs and specialist CMOs must look at developing collaborations to ensure that manufacturing technologies can continue to achieve the drug candidates and deliver the products on the scales required for the global demand.

Short peptide sequences, typically less than 25-30 amino acids in length, are significantly more economical to manufacture than longer peptides. On a large scale, these sequences are typically prepared utilising solution phase chemistry, achieving a lower manufacturing cost as compared to solid phase peptide synthesis (SPPS) methods which allow access to much longer peptide sequences.

The developments into SPPS using Fmoc chemistry have led to larger peptide molecules, and small proteins, being accessible by chemical synthesis on scales of multi-Kg and 100 Kg. The methodology of SPPS is based upon the first amino acid (at the C-terminus) being chemically bonded to an insoluble resin support. The amino acid residues are then chemically reacted sequentially on to the insoluble resin support, with all other reagents used in the synthesis being removed in solution away from the resin. The residues are added via a method of removing the Fmoc group that protects the amine function, and then adding a carboxyl-activated version of the next amino acid, forming the amide bond. Washing the insoluble support removes unbound molecules (reagents used in coupling chemistry).

Solid-phase cGMP peptide synthesis at Pepceuticals Ltd., using a batch-wise process.



There are two approaches to using SPPS, either in a batch-wise fashion, or in a continuous-flow fashion. Each approach has its benefits, dependant upon the stage in the drug discovery programme. Batch-wise synthesis, shown in the picture above, is more rapid for delivering a large number of peptides, such as for screening, however the quality of the peptide produced may be low, and lead to increased purification costs. Continuous flow presents higher purity peptides straight from the synthesis, and can reduce purification costs, however continuous flow can take longer to synthesise the peptide, and current automated equipment does not have the same capacity as that of batch-wise synthesisers.

More modern purification processes allow for larger-scale purifications of increasingly complex synthetic peptides, thus reducing the traditional 'bottleneck' in peptide manufacture.

Peptide Market

In the US, annual sales of peptide drugs exceeds $13 billion, representing 1.5% of drug sales globally. In addition, protein drugs such as therapeutic antibodies represent a larger share, with the combined biopharmaceutical market valued at over $70 billion. In Europe, Germany and the UK account for 63% of the peptide therapeutic market, with France, Italy, Scandinavia and Spain making up the rest of the major markets. With increasing biomedical research emphasis directed towards using peptides, the market is set to grow and broaden considerably over the coming years. In addition to the use of peptides as therapeutics, they have also found use acting as 'carrier-species' when conjugated to a small molecule or other drug substance. The uptake and high specificity of the conjugated peptide gives better delivery of the active substance, and often leads to a decrease in toxicity. This has brought renewed interest to established treatments, and there is significant opportunity to exploit the intellectual property which is still widely available.

Kilo-scale purification at Pepceuticals Ltd., under cGMP conditions.

Nutraceuticals, providing health benefits and prevention/healing of disease, has seen a respectable growth over the past few years. Supported primarily in markets where there is disposable income and an effort to maintain healthy living, nutraceuticals has seen a growth in North America of more than 6% for the period 2007-2011, with the protein and peptide section of dietary supplements expected to have a continued global growth for the foreseeable future. In 2011, peptides and proteins held the largest share of the dietary supplement market in North America, Africa and across the Middle East, as compared to vitamins and minerals, and herbal supplements. Increasing education and evidence about the benefits of such dietary supplements will continue to support the market, and its use of peptides/proteins is set to become more regulated, allowing opportunities for traditionally more pharma-oriented CMOs to expand their customer bases.

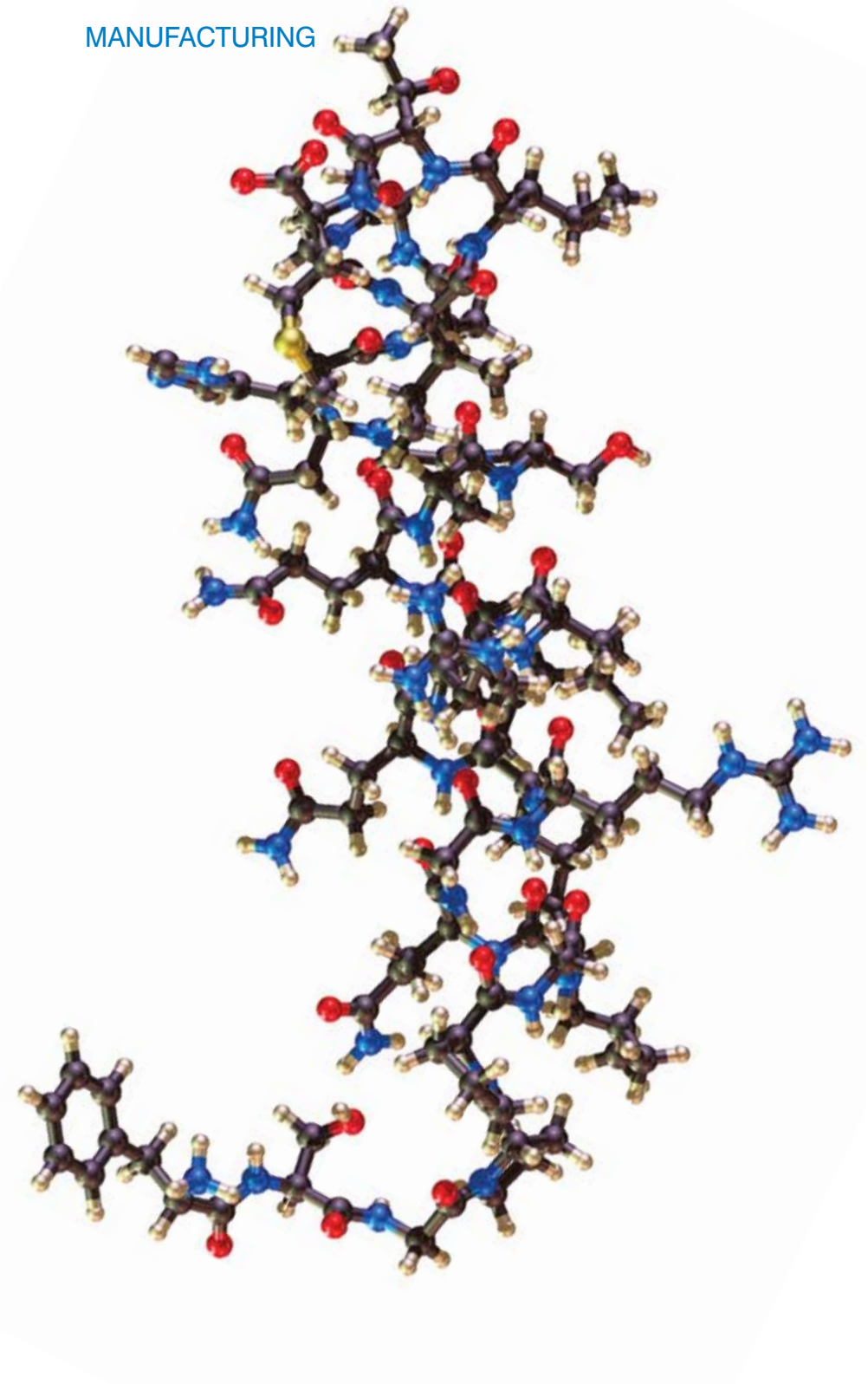
Finding a specialist CMO which can offer a range of pre-cGMP development techniques and methodologies is often a hard task, and then transferring this to a cGMP-capable facility with the right experience is time-consuming and costly. CMOs offering a complete service, or a range of key steps to support a drug discovery programme, are able to save unnecessary time-wastage and investments and give a peptide product which has been confidently transferred through the development stages. CMOs understand that a customer may expect their product, presented as a high quality material, at a low cost price, to be delivered shortly after a purchase order is issued. However, unfortunately this is not something that the CMO can completely achieve, and it is often that one or two of the demands must be sacrificed in order to present the product correctly. It can sometimes be easy to lose sight of the overall target for the drug discovery programme – to deliver a quality and reliable product, with a specified manufacturing process, in a good time-frame, and at an economically advantageous cost.

Looking to the Future

The employment of peptides in medical therapies and diagnostics, and as conjugates to other therapies, is set to increase, along with the range of disease areas that they look to combat. In parallel, the use of nutraceuticals, antibacterials, agricultural products, and cosmetics will continue to flourish. Peptides it seems have finally, over the millions of years of their existence, started to take their place at the forefront of research, and will continue to provide solutions to a wide range of applications for years to come.

Dr Kamal Badiani is Managing Director of Pepceuticals Ltd., a CMO specialising in the active substance manufacture of peptides and small molecules, based in Leicestershire, UK.

Kilo-scale purification at Pepceuticals Ltd., under cGMP conditions.



Pepceuticals Ltd., modern active substance manufacturing facility in Leicestershire, UK.

Pepceuticals Ltd., established in 1998, specialises in the manufacture of bioactive molecules for the life science industry, ranging from peptides to small molecules. The new manufacturing facilities based in Leicestershire offer custom manufacture of research-grade materials through to cGMP, in versatile laboratories recently audited by the MHRA (July 2011). Pepceuticals Ltd currently has the capacity to manufacture from mg up to multi-Kg of material. The facility comprises of eight state-of-the-art cGMP suites, covering the complete manufacturing process (Grade C, Class 10,000) through to fill/finish (Grade A, Class 100). The cGMP manufacturing is supported by laboratories dedicated to pre-cGMP process development, analysis and quality control. To complement the contract pharmaceutical drug development services, Pepceuticals Ltd offers a comprehensive bioanalysis service, utilising state-of-the-art equipment for detection, identification and quantification of biomarkers.

Waters Synapt High Accurancy Mass Spectrometer, capable of biomarker identification and characterization, protein sequencing and rapid sample analysis.

Dr Kama Badiani graduated with a Doctorate in Organic Chemistry from the University of St. Andrews in 1996. Since leaving St. Andrews Kamal worked within the pharmaceutical industry as a research chemist for two years before he founded Pepceuticals, in 1998. Under his leadership Pepceuticals soon became established as a manufacturer of synthetic peptides.

As an organic chemist, Kamal has developed the novel chemistry used today in Pepceuticals to produce/manufacture peptides, as well as the innovating new products that are currently in development. Over the last 14 years, Pepceuticals has grown to become one of the largest peptide companies in Europe, having moved to a new modern facility in Leicestershire, comprising of over 10,000 sq. ft of laboratory space. He has cultivated a reputation for outstanding quality and timely delivery. He believes that his team is fundamental to Pepceuticals' continued success.

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