

# The Future of Peptide Development in the Pharmaceutical Industry

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*A couple of weeks ago I was asked how long I thought the current interest in peptides as drug candidates would last before another class of molecule replaces them. My answer, based on the potential for new leads from genomics and the length of clinical evaluation, was "about another 20 years". I realized almost immediately that I have been saying that for at least the last 10 years. Perhaps it is time to re-think this one. Obviously, the interest in developing new peptide therapeutics is going to be around a lot longer.*

## The growing significance of peptide therapeutics

So what has happened in the last decade that indicates that peptides drugs have a long and secure future?

Well, first there is increasing amount of statistical evidence to support this<sup>1</sup>. Forty years ago, back in the seventies, the average frequency of peptides entering clinical trials was just over one per year. Ten and twenty years on, it was 5 and 10 per year respectively, and as we approach the end of this decade, it looks like the annual number will be close to 20. There are now about 60 approved peptide drugs that will generate annual sales of approximately US\$ 13 billion by the end of 2010. Although this only represents about 1.5% of all drug product sales the numbers are increasing dramatically with a current continuous annual growth rate (CAGR) between 7.5% and 10%. There are about 140 peptide candidates in the clinic and (our best guess) about another 500 – 600 in pre-clinical development.

However it is more than just the numbers. Peptide therapeutics are starting to show a maturity that reflects their potential for addressing a growing range of medical challenges. While about half of the peptides in clinical trials target indications in oncology, metabolic, cardiovascular and infectious diseases, the total range of therapeutic areas addressed encompasses a wide assortment of medical disorders from endocrinological lesions through to pain and hematology. A noticeable increase in the number of peptides targeting metabolic disorders has heralded a move towards longer and more complex molecules. There is also a willingness of both the pharmaceutical industry and its contract manufacturers to consider production scales of multi-100 kg and even tons using both solid- and solution-phase synthetic strategies.

Not that shorter peptides (<10 residues) are going away and the growing interest in peptide-based therapeutic vaccines is currently opening up a whole new market for such sequences. The manufacture of short sequences is significantly more economical than for longer peptides (>30 residues). Such sequences, if required in large quantities, are also often amenable to solution-phase approaches that offer scalability advantages over solid-phase chemistry.

The potential of peptides should be no surprise. Evolution has been honing the specificity of polypeptides for millions of years. Amino acid sequences – whether they are in peptides or in proteins – control and direct all aspects of cellular function and coordinate most intercellular communication. No other class of biological molecules offers the range of chemical diversity that peptides and proteins possess. They are nature's tool kit and the more we can use native peptides or closely related analogs, the safer and more specific the drugs at the physician's disposal. An advantage over small molecules is the fact that the structural relationships between peptide drug candidates and the physiologically active parent molecules that they were derived from substantially reduces the risk of unforeseen side-reactions. This is reflected in an over 20% probability of regulatory approval<sup>2</sup>, a rate which is double that of small molecules. In so far as they are composed of naturally occurring or metabolically tolerable amino acids, they are generally non-toxic and, as a result, toxicology studies often consume vastly more peptide drug substance than the subsequent clinical trials. Where side-effects occur, these are often related to dosage or local reactions at the injection site. In general, peptides do not cause serious immune responses, although this must always be taken into consideration, particularly for longer sequences.

## The impact of new technologies

Peptides have not always been the most popular candidates for drugs and it is worth asking why and what might have occurred to change the bias against peptides as pharmaceutical agents. For many years, peptide-based therapies were regarded as being limited to the treatment of hormonal lesions and later hormone-dependent cancer. Other indications were not obvious at the time. In addition, peptides – due to their chemical structure – were expensive and complicated pharmaceuticals to manufacture. They also often had exceptionally short half-lives making chronic administration problematic and costly. However, the main downside was the lack of oral availability. With a few notable exceptions, peptides are degraded to their component amino acids in the upper gut. Given the lack of patient compliance with drugs that require chronic self-injection – the exception being drugs for life-threatening diseases

such as Type I diabetes and insulin – wherever the pharmaceutical industry saw an oral alternative they opted against peptide (and protein-based) drug candidates.

In the late 1980s, the introduction of long-acting release (LAR) forms of peptides such as GnRH and somatostatin analogs encapsulated in biodegradable polymers that only required injection at extended intervals was a major step in increasing the acceptability of peptide drug substances. However, it was the advent of pharmaceuticals based on genetic engineering and recombinant technology that gradually, but radically changed the mindset of the industry. Paradoxically, these new technologies that many thought would make “peptide chemistry” - as a strategy for manufacturing peptides - redundant, actually succeeded in breaking down the main barrier to peptide drugs (lack of oral bioavailability) and caused a surge of interest in the chemical manufacture of peptides. Genetically engineered proteins offered a window to previously untreatable medical conditions and – with it – the industry accepted the need to develop drugs that could not be orally administered. It also initiated a massive drive to find alternative drug delivery platforms (to injection) that would find patient acceptability and compliance.

Moreover, recombinant technology as a tool for manufacturing peptides has not made the chemical synthesis of peptides redundant – far from it. There are a number of reasons for this. The first has to do with the nature of recombinant technology itself. Recombinant processes require substantial design and development before clinical manufacturing lots become available. The use and control of biological organisms and the associated genetic engineering require extensive quality management and regulatory control. Downstream processing is usually complex and customized to the specific product. In spite of the almost negligible costs of starting materials, the price of even the smallest scale GMP-grade manufacturing lot using fermentation of a recombinant organism is likely to exceed US\$ 1 million with a lead-time of over 1 year.

The second reason that chemical synthesis, in particular solid-phase procedures based on Fmoc-chemistry, is still the manufacturing procedure of choice for peptides has to do with changes in the peptide manufacturing industry. The last 15 years has seen a remarkable decrease in the cost of solid phase manufacturing – partly due to the cost of raw materials and economy of scale, and partly due to the technical improvements in chromatographic equipment and media. There is no doubt that solid-phase chemistry approaches are faster and less expensive for

manufacturing at up to a multi-10 kg or 100-kg scale and are more suited to early stage clinical development. In contrast to recombinant technology, solid phase chemical approaches require significantly less process development, use mainly generic chemical and purification procedures, and are less personnel intensive in terms of production, quality assurance and regulatory affairs. Chemical approaches also allow significantly more flexibility in design of analogs that require unnatural amino acids or non-proteogenic components.



However, there is no doubt that recombinant technology will play an increasingly important role in peptide manufacturing in the future because of quantity requirements. A number of peptides such as salmon calcitonin, human glucagon, human PTH (1-34) and human brain natriuretic factor, manufactured by recombinant technology, are already commercially available. Procedures are already being developed that allow the use of redundant codons to introduce unnatural amino acids into proteins and peptides, offering substantially more flexibility in the peptide sequences available to this technology.

Although many costs of chemical manufacture of peptides have decreased significantly in recent years, local labor and some solvent costs continue to rise, and the unit cost of goods is still a major component for drug manufacturers. One needs to be aware that the manufacturing cost of some peptide drug substances – particularly those above 30 amino acids in length – could represent significant challenges to the final cost of goods of the drug product if they have to be given chronically at a high dose (>100 mg/day). If this is where you plan to go, you would be well advised to do some careful calculations before committing to long-term development. Economical commercial processes can be developed for most peptides, but significant, maybe multi-million dollar investments may be required along the way to achieve that goal. Fortunately, many of the longer sequence candidates under development are exceptionally potent with daily doses in the microgram range.

In passing, one should mention that there are still some peptides on the market that are isolated from animal sources, but their numbers are dwindling. There is also some re-emerging interest in using enzymatic procedures (reversed proteolysis) to synthesize specific sequences. The use of free amino acids as opposed to Fmoc-derivatives as starting materials has great economic appeal.

### Current challenges

One of the challenges facing the manufacturers of both peptide drug substances and peptide drug products today is the inability of regulatory authorities on different continents to come up with a harmonized set of guidelines that define what level of peptide impurities can be present in peptide therapeutics. It is a challenge for two reasons. First, no-one in the business is totally sure what is expected of them, and – secondly – designing manufacturing processes to produce a higher quality product than is required adds significantly to the cost of goods, possibly to the point where an effective drug loses its economic viability.

The underlying issue is that the term “peptide” or even “complex peptide” is too vague to have meaningful regulatory usage. Peptides range from sequences containing only 2 amino acids, which are obviously “small molecules”, to sequences of up to 100 residues, where historically biochemists arbitrarily decided that anything larger should be called a protein. That delineation is even fuzzier today where – in deference to insulin as the long-standing border line between recombinant and chemical synthesis – sequences longer than 50 amino acids are often referred to as “small proteins”.

The two extremes – dipeptides at one end and 50 to 100-meric polypeptides at the other – represent peptidic molecules of vastly different complexity with significantly divergent physical and chemical properties, as well as different abilities to adopt secondary and tertiary structures. Small molecules and biologics are justifiably treated as different classes of molecules by the regulatory authorities. Peptides, which span both categories, cannot be assigned *in toto* to either. Given the mainly non-toxic nature of peptides, an impurity profile guideline that is based on “dose” and “indication”, or on the factual data generated in the tox studies, might be more appropriate for those peptides that do not fulfill the requirements for small molecules. This is perhaps a too simplistic approach, and certainly any extrapolation of data from tox studies can only be justified if the identical manufacturing process is used for both tox and clinic. Nevertheless, the lack of harmonized guidelines needs addressing.

An unfortunate spin-off of the lack of clear guidance for setting impurity profile specifications is that many drug product manufacturers adopt the most conservative and stringent limits for peptides (effectively using small molecule API guidelines for complex peptides) in order to ensure that “no regulatory issues” arise. If one considers that most peptide drugs have exceptionally low toxicity and are administered at doses between 50 µg and 50 mg, requiring limits for individual, unidentified impurities of less than 0.1% constitutes an element of “overkill”. While such caution may be warranted for some products, for many it simply results in an unnecessary additional cost of goods.

While few companies can – at the start of product development – specify the final dose or will be prepared to commit decisively to the commercial quantities that will eventually be required, a “best guess” of the commercial scale of manufacture, the time-line to achieve this and the anticipated cost of goods helps CMOs design appropriate manufacturing processes. As a project moves from the multi-100 gram to the multi-10 kg scale and beyond, the process will almost certainly have to evolve or change. The failure to recognize this early in a project may result in progressing past a point of no return where changes are not acceptable from a regulatory or economic standpoint. The choice of counterion is an example. The solubility and stability of peptides can be influenced by their salt form and the initial choice of counterion may be disadvantageous for large scale manufacture. However, from a regulatory standpoint different salt forms of a peptide are considered to be different drug substances and changing from one to another may, therefore, be challenging.

For biotech and emerging pharmaceutical companies with their limited budgets, one of the most challenging issues today must be finding the correct balance between expediency and due diligence. Many small biotech companies do not have quality assurance or regulatory affairs departments with adequate experience with peptides. Every CMO knows customers that expect their products to be delivered shortly after the purchase order is issued, if not before. Right quality, right time and right price (equating to high purity, expedited delivery and low unit cost respectively) are commonly cited goals at the start of most projects. Unfortunately these three demands are not mutually compatible and one usually has to be sacrificed to achieve the other two. In the hurry to move forward, it is often easy to lose sight of the ultimate goals, which should be (a) an expedient approval, (b) a robust, scalable and economic process, and (c) a profitable drug product. Particularly for longer peptides, the urge to push forward quickly, without duly diligent characterization of the active pharmaceutical ingredient, can result in disaster. Typical issues are the failure to detect impurities which cannot be removed chromatographically and then, as a consequence, may require an alternative synthetic approach. Many analytical HPLC systems fail to resolve enantiomeric forms or other isomers (e.g.  $\beta$ -aspartyl transformations) of peptides, particularly if the sequences are long. If such co-eluting impurities arise due to degradation of the peptide during storage, the peptide may continue to appear to meet the HPLC purity specifications, but lose its pharmaceutical potency. Such impurities are often missed unless the analytical method is specifically designed to detect them.

For peptide API manufacturers one of the current challenges is matching equipment and other resources to meet customer requirements. Most clinical trials of peptide APIs involve annual quantities between 10 grams and 10 kilograms. While most of the larger CMOs are equipped to handle several parallel campaigns at this scale, they are not all so well equipped to make annual quantities of 100 kilograms to a ton. There are very few projects that routinely exceed 100 kg per year at a single site, the most commonly cited being those of Fuzeon, Eptifibatid and Bivalirudin. The acquisition and maintenance of equipment for this scale of manufacture is difficult to justify if it is sitting idle for most of the year.

The use of large equipment does not always equate to economy of scale and certainly exposes both sponsor and contract manufacturing organization (CMO) to enhanced risk if equipment fails. Working at larger scale is usually associated with longer hold times during or between process steps, conditions which can favor degradation or aggregation. It is not difficult to make a case for the use of more modest equipment organized in tandem or parallel configuration. Whatever the process that is eventually chosen to manufacture at a multi-10 kg or multi-100 kg scale, it will probably have been through significant development and may not resemble the process of the first GMP lots at all. It may not even be based on the same technology, e.g. if the switch to recombinant fermentation is made.



## Perspective for the future

So what can we expect from peptide therapeutics in the future?

We can expect the number of peptides entering clinical trials to grow. We can expect the overall complexity of peptide APIs, their potency (by design) and their specificity (including the ability to target specific organs and cells) to increase. The use of peptides conjugated to PEGs, carbohydrates, antibodies and other proteins will become more frequent. Peptides will not only be used as the active ingredient of new drugs, but as “add-ons” to other pharmaceutical agents to direct them to their targets, to ferry them across cellular membranes, and to modify their biological action.

We can expect the range of medical indications that peptides address to grow. We can expect peptide-based antimicrobial peptides to find commercial use. Almost certainly peptides will find increased usage to treat obesity, metabolic syndromes and Type 2 diabetes. The use of membrane-penetrating peptides will increase the number of intracellular targets. Peptides will be used to address currently “undruggable” targets.

We can expect a massive upsurge of interest in peptide therapeutics to occur once a delivery platform, that can deliver short-acting peptides efficiently into the bloodstream, has been developed. This is not a question of “if it happens”, but “when”. While an oral route to obtain maximum compliance would be most desirable, there are a number of non-invasive (pulmonary, nasal) or minimally invasive (transdermal) devices in development that might provide suitable and, in terms of efficacy, more attractive alternatives.

We can expect the introduction of novel formulations and excipients to stabilize peptides at room temperature. We can expect the efficacy of long-acting formulations to improve and to enable smaller quantities of peptide drug products in the body to maintain activity over longer periods of time. At the same time we should not forget that many peptides do not exert their pharmacological action only when maintained above a threshold concentration in the body. Indeed, most peptides exert their biological function by acting as signals; “switching off” can be as important as “switching on”. This requires a combination of pulsatile administration and the ability of the body to degrade the peptide. One can anticipate that computerized transdermal or implanted delivery devices may eventually be able to fulfill this function. Coupled with physical or chemical targeting devices, the concept of total temporal and chemical repair of hormonal and other “signal function” lesions may become a reality.



Although one can never rule out the introduction of new chemistry, solid phase peptide synthesis based on Fmoc-chemistry will probably continue to be the “first choice” for the manufacture of most peptides for some time to come. Changing established approaches of manufacturing is significantly more difficult in a GMP environment than in the research laboratory. Manufacturers’ raw materials need to be economically affordable commodities available for all scales of production. It is also difficult to draft contingency (back-up, secondary supplier) plans for proprietary manufacturing technologies. We will continue to see a steady increase of new amino acid derivatives, of “exotic” amino acids and reagents. New strategies for coupling peptide fragments may contribute to peptide chemistry advancing regularly into the territory of pharmaceutical manufacture of small proteins without the risk of uncontrollable impurity profiles. Perhaps even more likely, we will see the borders between peptide chemistry, recombinant fermentation, enzymatic synthesis and bio-organic chemistry overlap and merge, and become one set of tools for the design of large scale manufacturing processes rather than being different branches of contract manufacturing.

One would hope that, as experience with peptides grows, the regulatory directives will provide more guidance to industry as to how specifications for impurity limits should be set. Without that guidance, many potential peptide drugs may fall by the wayside due to the technical or economical inability to meet specifications that are excessively tight.

There are also many peptide-associated issues where new regulatory approaches are needed. Vaccine “cocktails” is one. It is difficult to conceive how the current regulatory expectations for peptide APIs for therapeutic usage (e.g. limits for impurities) can be applied to small gram quantities of the several peptide components of a vaccine product, which is not administered chronically, and still be economically viable. Others include polydispersed peptides (e.g. poly-lysine) or peptides attached to polymers which currently pose significant challenges to analytical characterization. However, one should not forget that Copaxone, which has the highest current sales for any peptide therapeutic, with worldwide sales of over US\$ 3 billion, falls into the category of polydisperse hetero-polypeptides, and is clear proof that a good drug can “make it” whatever the analytical or regulatory challenges.

As a final comment, we should mention that we – as peptide contract manufacturers – often take a retrospective look at those peptide therapeutic candidates that have made it and those that have not. There is really no way of knowing at the start which ones will be successful. The only facts we know for sure are that peptide therapeutics are continuing to flourish and that their foreseeable future is secure.

<sup>1</sup>For anyone wanting to view statistics about peptide therapeutics in more detail, the Peptide Therapeutics Foundation publishes a bi-annual report “Development Trends for Peptide Therapeutics” and you should visit their website at <http://www.peptidetherapeutics.org/peptide-therapeutics-foundation.html>

<sup>2</sup>There is a 10 - 12 year lag between a peptide drug candidate entering clinical trials and its potential approval. The probability of regulatory approval can be obtained by analyzing the individual approvals in a fixed time period and comparing that with the number of peptide drug candidates entering clinical trials 12 years earlier. By analysing consecutive time periods an extrapolated probability can be calculated<sup>1</sup>.

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