By Dave Robinson, CEO
New England Peptide, LLC

Never has there been a more exciting time to be working with peptides. The compilation of thought-provoking articles from the industry's leading solution providers in this inaugural issue of PharManufacturing conveys a renewed focus on peptides and their potential.

All segments of the global peptide market are thriving, from therapeutics to vaccines to drug delivery to diagnostics to cosmetics. Growth drivers such as improved drug delivery methods and enhanced manufacturing capabilities are fueling the surge in peptide use, while novel therapeutic applications owing to a peptide's low toxicity and high potency are entering the pipeline at a rapid pace.

The $6 billion (U.S.) worldwide peptide drug market alone is projected to grow to $10 billion by 2010. While the United States plays host to the largest share of the work in peptide research and development, significant research networks exist in Europe and Asia as well.

With the rapidly growing list of applications, peptides are touching the lives of people in all walks of life and in all corners of the world. As people harness the power that peptides possess, this list will lengthen.

✓ Oncology/cancer
✓ HIV/AIDS
✓ Cardiovascular
✓ Diabetes
✓ Metabolic disease (e.g., diabetes)
✓ Infection
✓ Central nervous system
✓ Dermatology

Yet with so much opportunity, so comes the challenges of navigating the wide array of sourcing options for quality peptides. The peptide supply chain is highly fragmented with many small players serving specific niches, so companies and organizations must turn to multiple providers to serve all peptide needs. Multiple questions arise throughout a project's lifecycle: Which peptide provider should one trust with such a critical ingredient for success? Is location relevant? What is a fair price to pay? What is the right technology for the project?

The cost of poor quality is too great to place an uninformed or risky bet on peptide source. Projects – and entire organizations – can fail months or years down the road if peptide quality is compromised. A natural trend toward working with increasingly difficult sequences compounds the quality issue. Accordingly, companies are looking beyond their internal resources for peptide production.

The good news is that attractive solutions to these challenges do exist in the market today, and PharManufacturing is here as a key resource to help individuals along the way. The right choices and decisions will help position one for market success and for helping millions of people worldwide.

By Dave Robinson, CEO, New England Peptide, LLC

NEP
New England Peptide
Committed to Cures

New England Peptide designs and manufactures custom peptides and polyclonal antibodies primarily for the therapeutic, vaccine, cosmetic and diagnostic markets. NEP collaborates with scientists at biopharma companies, academic institutions and government agencies around the world and has begun preparation to offer pharmaceutical-grade peptides to position the firm as a single source for all peptide needs.

Synthetic Peptides
A Bench Reference

New England Peptide

By Sam Massoni, Dr. Rangaraju Naramishachar and Dr. TK Prakasha

Synthetic peptides are an excellent tool in biopharmaceutical research. Their vast potential is best realized by following a few simple guidelines for peptide design, use, handling and storage.

Sequence Selection

When selecting a peptide sequence, be mindful that design for manufacturability is an important factor since some sequences can cause production delay or failure. When possible, follow these guidelines to aid in synthesis success.

Avoid problematic amino acid locations inside the sequence:

- ✓ A Glutamine (Q) residue at the N-terminus of the peptide may undergo spontaneous transformation into a pyroGlutamic residue. To prevent this transformation, acetylate the N-terminus, add another amino acid N-terminally to the Q, or remove the Q altogether.
- ✓ Cysteine (C) and Proline (P) have proven to be problematic when located at the C-terminus of the peptide. To prevent issues, amidate the C-terminus, add another amino acid C-terminally to the C or P, or, eliminate the C or P altogether.
- ✓ An Aspartic Acid (D) residue located anywhere in the peptide can spontaneously transform into an Aspartamide residue regardless of its location in a sequence.

Watch out for certain amino acid combinations:
Aspartic Acid/Glycine (D/G) pairings or numerous Threonine (T), Cysteine (C), Leucine (L), Valine (V) or Isoleucine (I) residues in a row can be troublesome. Where possible, avoid such combinations in sequence selection in order to position a peptide for production success.

Recreating the Natural Environment

Peptides are created to mimic proteins or the cleavage products of proteins. When proteins are cleaved *in vivo*, they have naturally occurring free unprotected termini. Therefore blocking the termini is not necessary for *in vivo* cleavage. However, when the sequence is not a known cleavage product, blocking the termini is necessary in order to mimic the peptide bonds normally found in the parent sequence.
Use the following rules:

- ✓ If the sequence is C-terminal, block the N-terminus by acetylation
- ✓ If the sequence is N-terminal, block the C-terminus by amidation
- ✓ If the sequence is internal, block both ends with acetylation and amidation

Verifying Peptide Quality

Since production and quality control ("QC") techniques vary by peptide producer, always verify the peptide quality prior to using the peptide. At a minimum, the **QC check** should include a detailed review of the quality documentation that accompanied the peptide. There are three key documents to review.
Mass Spectrometry – The mass spec confirms the peptide identity by reporting the molecular weight of the sample. The resulting data should be within 0.1% Daltons of the theoretical molecular weight.

HPLC – The HPLC trace reports peptide purity. It is important to make certain that the HPLC data includes gradient time and percentage. A gradient faster than 2% per minute may mask impurities, making the peptide appear more pure than it actually is.

Certificate of Analysis – A certificate of analysis should detail the raw materials used (including corresponding manufacturers and lot numbers), instruments, assay details and QC signatures. Proceeding with research without this level of traceability and reproducibility is not recommended.

Selecting Target Purity

Selecting the appropriate peptide purity is assay dependent. The following are some basic research-driven guidelines:

Application	Non-sensitive screening assays	Immunogen grade	Receptor/ligand, bio-assay or cell studies	Structural studies
Recommended purity	Crude or >75%	polyclonal >85% monoclonal >95%	>95%	>98%

Reconstituting a Peptide

Reconstituting a peptide can pose challenges, but proper planning can eliminate common problems.

- ✓ Bring frozen or refrigerated peptides to room temperature in a desiccated chamber to avoid water absorption.
- ✓ Always begin by reconstituting a small amount of peptide before committing the entire lot.
- ✓ Use sterile water or sterile filtration. If there are any Methionine (M), Cysteine (C), or Tryptophan (W) residues, use oxygen free solvents to prevent oxidation. Avoid reconstituting a peptide in a buffer, like PBS, since salts hinder solubility.
- ✓ A solubilized peptide is completely clear. No flecks or cloudiness should be present.
- ✓ Should the peptide not go into solution, re-lyophilize the peptide and begin again or centrifuge or filter the peptide to remove insoluble particles.

Amino Acid Characteristics	**Recommended Solvent**
Hydrophilic residues (KRHDEPN)	H_2O*
Hydrophobic residues (AVLIMFWP)	Low solubility in aqueous solvents; are soluble in organic solvents (DMF, DMSO, TFA, Acetonitrile)

*If the peptide does not go into solution completely, calculate the pI of the peptide and add either 0.1N acetic acid or 0.1N ammonium acetate to adjust pH away from the pI until solubilized. If the peptide still does not go into solution, try organic solvents such as DMSO, Acetonitrile or DMF.

Properly Storing a Peptide

Proper storage of a peptide can prevent bacterial degradation, secondary structure formation, oxidation and other potential degradation for several years. Peptides are most stable in their lyophilized form at -20° C or colder in a sealed container containing desiccant. If peptide must be stored in solution, ensure pH is in the 3-6 range and then aliquot peptide into usable sizes to prevent damage from multiple freeze/thaw cycles. Cysteine (C), Methionine (M), Tryptophan (W), Asparagine (N) and Glutamine (Q) are most sensitive to degradation in solution.

	Duration	**Storage Recommendation**
Long-Term Storage	3 months to 5 years	Lyophilized powder, frozen and desiccated, -20° C or colder
Short-Term Storage	0-3 months	Frozen liquid (-20° C) or refrigerated lyophilized powder

In summary, follow the design and use guidelines detailed above to harness the potential of peptides in biopharmaceutical research.

About the Authors:

Sam Massoni, senior scientist and founder of New England Peptide, is a career peptide chemist who continues to lead the industry in new directions.

Rangaraju Naramishachar PhD, a senior scientist at New England Peptide, is a 25 year veteran of the peptide industry who has led production teams and trained peptide chemists worldwide.

TK Prakasha - After focusing the early portion of his career in R&D at DuPont, TK took on new product development and business development responsibilities at Borregaard Synthesis. He currently drives business development at New England Peptide.

All production-related advice is based on FMOC solid phase peptide synthesis production methods.