Peptide Handbook

A Guide to Peptide Design and Applications in Biomedical Research

First Edition

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- **Diverse Delivery Options** help customers plan their peptide-based research according to their time schedule and with peace of mind.
- **ArgonShield™ Packing** eliminates the experimental variation caused by oxidization and deliquescence of custom peptides through an innovative packing and delivery technology.
- **Expert Support** offered by Ph.D.-level scientists guides customers from peptide design and synthesis to reconstitution and application.

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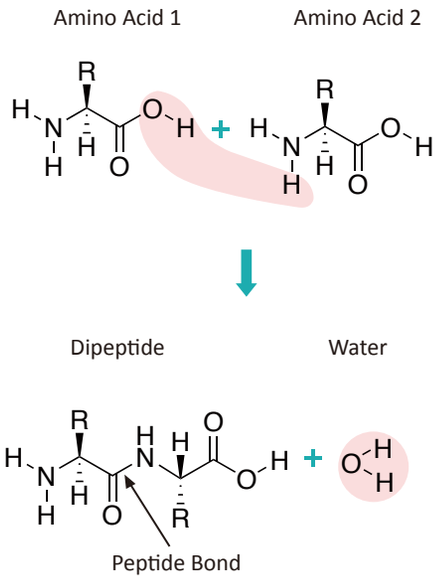
Chapter One

The Universe of Peptides

Molecular Structure

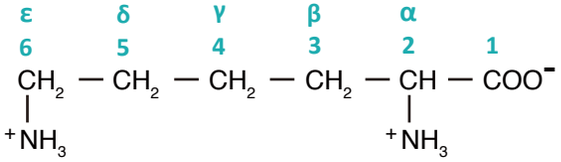
The word peptide, derived from the Greek word *“peptós/digested,”* refers to a chain of amino acids (AAs) linked together via amide or peptide bonds. The formation of the covalent peptide bond is an example of a condensation reaction, which generates water from the combination of the α-carboxyl group of one AA and the α-amino group of another. An AA unit within a peptide chain is called a "residue”. A peptide can be as short as two residues with only one peptide bond (named “dipeptide”) or as long as several residues forming a continuous and unbranched peptide chain (named “oligo-peptide” if containing <20 AAs or polypeptide if containing >20 AAs). Proteins are comprised of many peptide chains and sometimes the terms “polypeptide” and “protein” are used interchangeably. There are several different conventions for making a distinction between the two, but in general, molecules referred to as polypeptides have molecular weights (MWs) below 10,000 Daltons or less than 50 AAs, and molecules above these limits are considered as proteins (Lehninger *et al.)*

Peptide Bond Formation



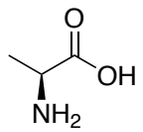
In a peptide chain, the AA at the end with a free α-amino group is called the "amino-terminal" (N-terminal) residue, whereas the one at the other end with a free carboxyl group is called the "carboxyl-terminal" (C-terminal) residue. The backbone carbon before the carboxyl carbon in an AA is called the "alpha carbon" (Cα) or "chiral center", where different side chains attach. Depending on the position of carbon atoms relative to Cα, the remaining carbons are named with Greek letters of β, γ, etc.

Numbering Carbon Atoms in an AA

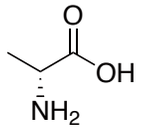


Due to the possibility of forming two different enantiomers (stereoisomers) around the central carbon atom, all AAs except Glycine can exist in two isomeric configurations of D (dextrorotatory; right-handed) and L (levorotatory; left-handed). Until recently, most AAs synthesized by eukaryotes were reported to be in the L-isoform, whereas D-isoforms were predominantly found in bacterial cell wall proteins or synthesized chemically. However, due to latest developments in sensitive analytical methods, free D-amino acids are shown to be present in the nervous system and venom of animals as well (Kiriyama & Nochi).

Amino Acid Chirality



L-alanine



D-alanine

Characteristics

Each peptide has its own unique structural and biochemical characteristics, such as an isoelectric pH (pI) and ionization behavior. These features are derived from the peptide's AA components. Therefore, the overall characteristics of a peptide can change depending on the quantity and type of each AA within the chain.

For example, the acid-base behavior of a peptide is defined by the nature of the free and bound AAs in its chain. The terminal α-amino and α-carboxyl groups sitting at opposite ends of the chain as well as the side chains (R groups) of some AAs can ionize and affect the titration curve. Similarly, due to the positioning of the chiral carbon and stereoisomer configurations in all AAs except Glycine, the newly formed L or D isomer of AAs can add to their unique characteristics. Ultimately all these characteristics influence how a given peptide behaves in a biological system.

Formation of Acid or Base from an Amino Acid

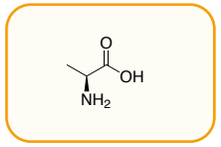
Positive Ion

In acidic conditions: acting like a base.

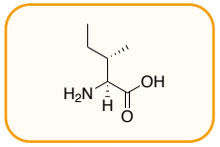
Negative Ion

In basic conditions: acting like an acid.

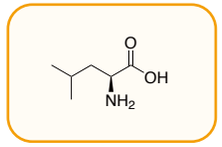
Common Amino Acids



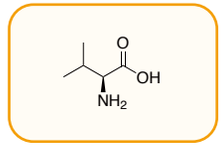
Alanine A
MW: 89.09
IP: 6.00



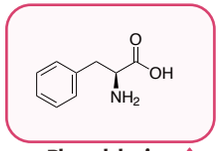
Isoleucine I
MW: 131.17
IP: 5.94



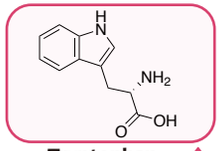
Leucine L
MW: 131.17
IP: 5.98



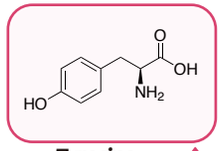
Valine V
MW: 117.15
IP: 5.96



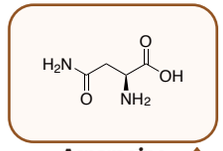
Phenylalanine F
MW: 165.19
IP: 5.48



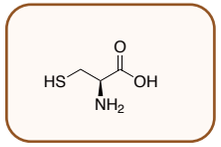
Tryptophan W
MW: 204.23
IP: 5.89



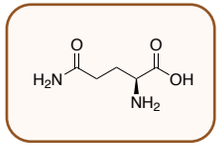
Tyrosine Y
MW: 181.19
IP: 5.66



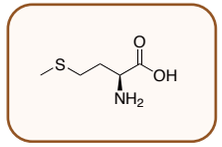
Asparagine N
MW: 132.12
IP: 5.41



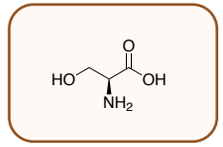
Cysteine C
MW: 121.16
IP: 5.02



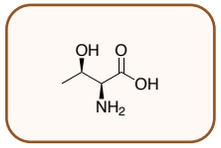
Glutamine Q
MW: 146.15
IP: 5.65



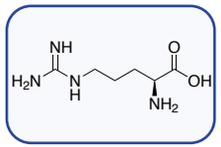
Methionine M
MW: 149.21
IP: 5.74



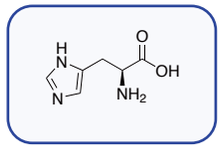
Serine S
MW: 105.09
IP: 5.68



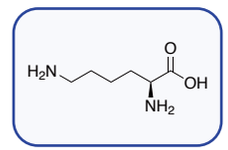
Threonine T
MW: 119.12
IP: 5.64



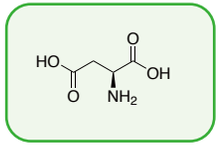
Arginine R
MW: 174.2
IP: 11.15



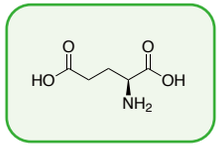
Histidine H
MW: 155.16
IP: 7.47



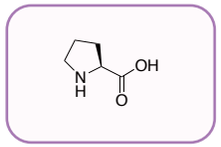
Lysine K
MW: 146.19
IP: 9.59



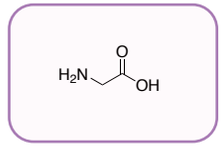
Aspartic Acid D
MW: 133.1
IP: 2.77



Glutamic Acid E
MW: 147.13
IP: 3.22



Proline P
MW: 115.13
IP: 6.30

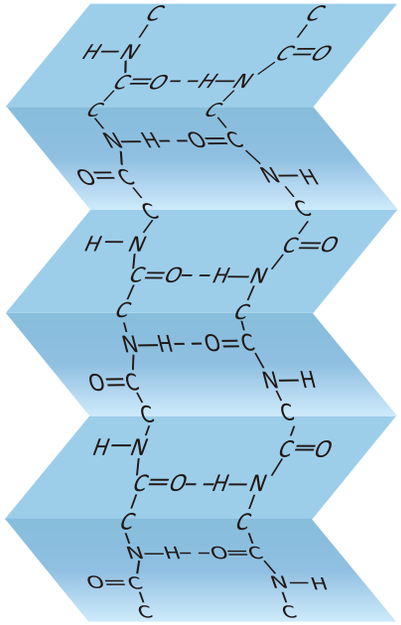


Glycine G
MW: 75.07
IP: 5.97

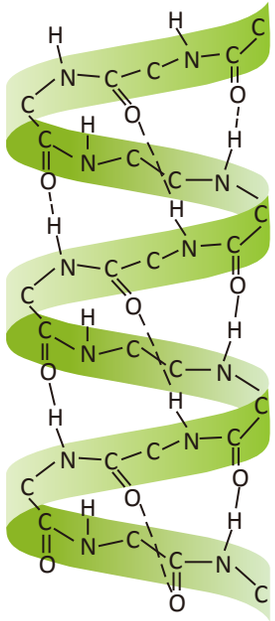
- Aliphatic amino acids with hydrophobic side chain
- Aromatic amino acids with hydrophobic side Chain
- Amino acids with neutral side chain
- Amino acids with positive charged side chain
- Amino acids with negative charged side chain
- Unique amino acids

MW: Molecular Weight IP: Isoelectric Point

Secondary Structure of Peptides



β-sheet



α-helix

GenScript's **Peptide Calculator** can help you determine the chemical formula and MW of your peptide of interest.

Categories and Biological Functions

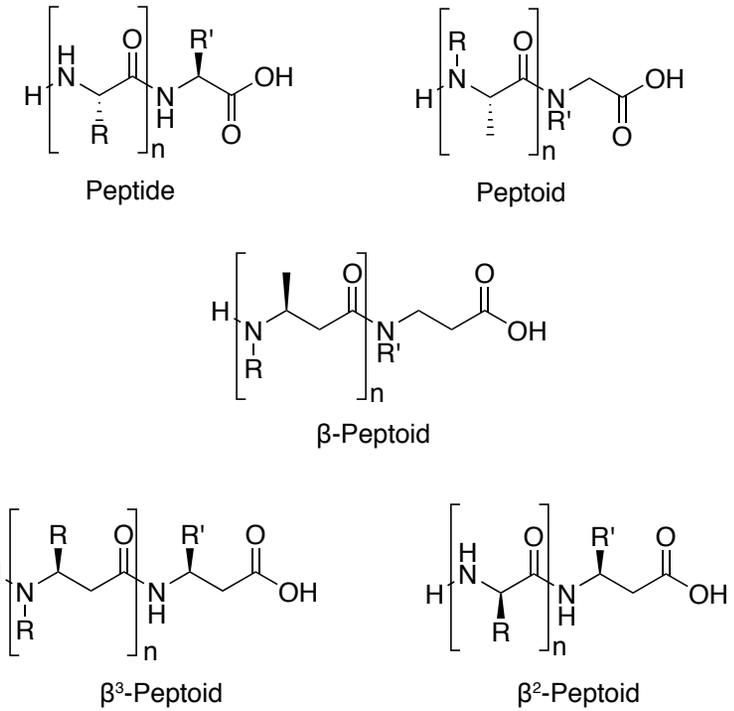
The 7,000 naturally-occurring peptides are present in all organisms and have extensive roles in the physiology of microorganisms, plants, animals, and humans. To better study and use peptides, they can be classified using different methods. The function of peptides as hormones, growth factors, ion channel ligands, neurotransmitters, and immune system components in biological systems is the basis of one method of peptide classification. Another approach is based on the way natural peptides are produced. Each method of classification may overlap with another and can be field or application specific.

Major Classes of Peptides

Class	Source	Application/Function
Ribosomal Peptides	mRNA translation	Antibiotics, Hormones, Signaling peptides, Bacteriocins
Non-Ribosomal Peptides	Non-ribosomal peptide synthetases	Toxins, Cytostatics, Siderophores, Pigments, Antibiotics, Immunosuppressants
Peptones	Enzymatic digestion or acid hydrolysis of natural products	Ingredient in bacteria and fungal growth media
Peptide Fragments	Enzymatic degradation of laboratory samples	Identification or quantification of the source protein
	Degradation of forensic or paleontological samples by natural effects	

The intrinsic characteristics of natural peptides, such as instability and proteolytic degradation limit their medicinal application. To overcome this limitation peptide analogues or "peptidomimetics" have been developed to mimic the biological actions of peptides without undesired restrictions (Olson). Peptidomimetics are synthesized by modification of an existing natural peptide or through introducing novel structural changes that render resistance to proteolytic degradation, increase thermodynamic stability and provide the capability to pass through the plasma membrane.

Peptide vs Peptidomimetics

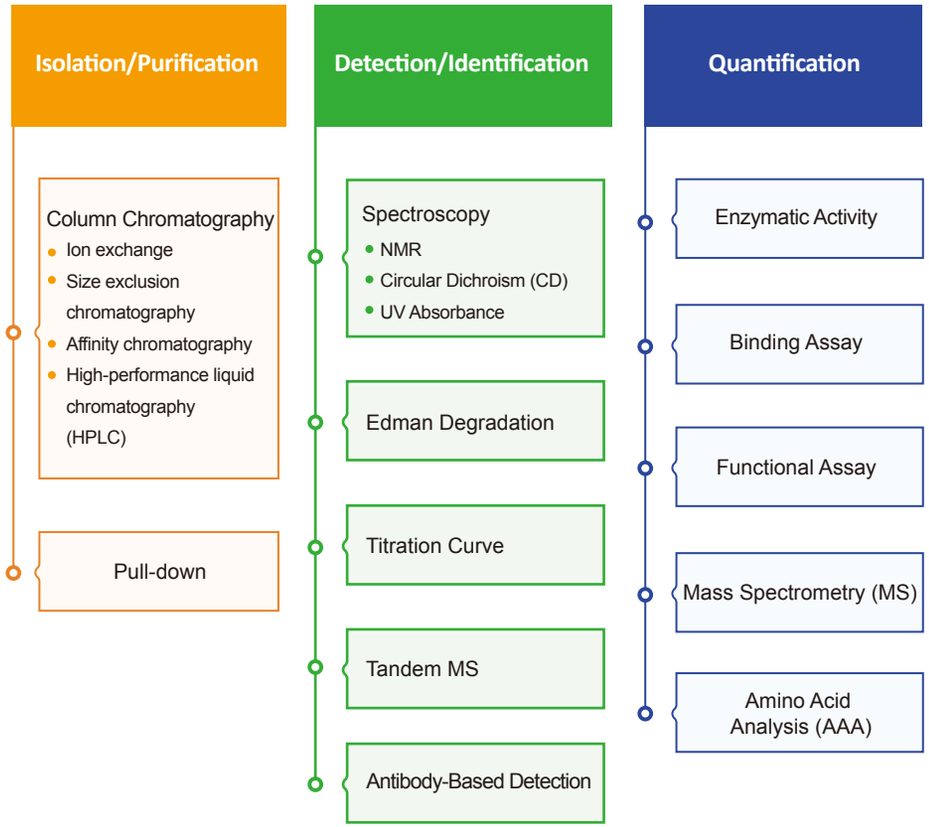


Adapted from Olson, 2010.

Analytical Methods

Proper synthesis, characterization, and application of peptides require appropriate analytical methods. A variety of methods are used in isolating, purifying, detecting, and quantifying all types of peptides. However, a few are more specific to the nature of a peptide and downstream applications. Furthermore, some methods are multi-functional in that they can be used for two or more analytical purposes. In recent times, many of these methods have been coupled with fluorescence capabilities for improved data collection and analysis.

Methods for Peptide Analysis



Chapter Two

Application of Peptides

Similar to the endless structural possibilities in a peptide chain, the application of peptides are vast and ever-growing. From research applications in microorganisms, plants, and animals to household use of antimicrobial wound dressings and antidiabetic drugs, peptides have left their prominent mark in today's world. In this section, we will briefly review the main categories of peptide applications to help enhance the current application of peptides in your research and also help you develop novel solutions and methods using synthetic peptides.

Research in Structural Biology

One of the earliest and most common applications of peptides is in biochemical research where secondary structure of native proteins are investigated *in vitro*. In such studies, purified or synthetic peptides are subjected to various analytical methods, such as nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography, surface plasmon resonance, circular dichroism (CD), microscopy, atomic force microscopy, fluorescence imaging and electron paramagnetic resonance. Depending on the nature of each technique, different quantities of peptides in simple or modified forms are utilized. Recent advancements in synthesizing peptides with modifications that replicate the native molecule or allow easy detection have enabled structural biologists to collect valuable information that is useful for other peptide applications, such as development of effective therapeutics.

Custom peptides synthesized by GenScript enabled Pustovalova *et al.* to use NMR spectroscopy for studying the structure of a DNA polymerase:

The C-terminal domain of human Rev1 contains independent binding sites for DNA polymerase H and Rev7 subunit of polymerase Z. FEBS Letter; 2012.

Research in Disease Pathogenesis

Understanding disease pathogenesis requires time, effort and access to reagents and tools for *in vitro* and *in vivo* studies. This endeavor is more difficult where the nature of the disease renders its *in vitro* study impossible unless new technologies can assist. Neurodegenerative diseases belong to such group of pathologies. In general, the hallmark of such diseases is the abnormal aggregation of molecules due to improper molecular processing. For example, main characteristics of Alzheimer's disease are extracellular aggregates formed by beta-amyloid peptides and intracellular neurofibrillary tangles consisting of Tau proteins (Glenner & Wong; Grundke-Iqbal *et al*.). The intrinsic properties of these aggregates stemming from peptide's hydrophobicity interfere with *in vitro* experiments. However, with the application of advanced synthesis and purification methods, neurodegenerative-specific custom synthetic peptides can conveniently be used for research in disease pathogenesis. A good example is the click peptide synthesis technology that allows for the retention of physicochemical and biochemical peptide properties in *in vitro* settings (Sohma *et al.*). By simply changing the pH, the synthetic peptide quickly converts to the native form with less aggregation and higher solubility. Click peptides can be used in the design or testing of aggregation inhibitors as well as characterization of other molecules involved in the aggregation process towards developing therapeutic drugs or vaccines.

Custom peptides synthesized by GenScript helped Monasterio *et al.* develop a novel catalytic amyloid peptide:

Development of a novel catalytic amyloid displaying a metal-dependent ATPase-like activity. BBRS, 2016.

GenScript's comprehensive **β-amyloid Peptide Research Toolbox** is enabling scientists to find a cure for neurodegenerative diseases.

Generating Antibodies

Generation of custom antibodies since the late 1800s to this day has come a long way. Use of the serum from a diseased animal as the main source of an immunogen is greatly replaced by two more specific methods. The first approach involves the use of the full length protein in either native, recombinant, fusion or even gel-extracted form as an immunogen. The second relies on careful selection of short, immunogenic sequences or epitopes of the native protein as the immunogen. Advancements in the synthesis and delivery formats of custom synthetic peptides have markedly increased the application of the second approach due to a plethora of advantages (Lee *et al.*). Synthetic peptides delivered in array or library formats can increase the efficiency of antibody epitope mapping and validation for finding the most "immunogenically fit" peptide. Such unique peptides are then used for generating cost-effective and more specific poly- or monoclonal antibodies for *in vitro* and *in vivo* settings. These antibodies have wide applications as reagents in biological assays or therapeutics in medicine. Successful application of monoclonal antibodies in cancer treatment, specifically, has revolutionized the field of precision medicine (Firer & Gellerman).

Advantages of Peptide Immunogens

- Less cross-reactivity
- Ease of synthesis
- Flexibility in antigen design
- Cost-effective manufacturing
- Fast turnaround time
- Good alternative for hard-to-generate protein immunogens
- Targeting post-translationally modified molecules

Custom peptides synthesized by GenScript helped Quiñones-Parra *et al.* to discover variations in T-cell immunity to the influenza A virus:

Preexisting CD8+ T-cell immunity to the H7N9 influenza A virus varies across ethnicities. PNAS, 2014.

GenScript's **Peptide Antigen Database** is a collection of optimal peptide antigen sequences predicted by our proprietary **OptimumAntigen Design Tool** for generating the most effective antibody.

Vaccine Development

In 1798 by using cowpox *(Variolae vaccinae)* to inoculate humans against smallpox, Edward Jenner coined the term "vaccine" (Riedel). Although similar practices were dated back to the 12th century, it was not until 1900s when the development and use of vaccines became mainstream. Since then the main application of vaccines have been in disease prevention, but in recent years vaccines have been widely used in therapeutic medicine as immunotherapies in oncology and rheumatology.

Depending on the nature of the pathogen and disease as well as practical considerations, a variety of approaches are used to generate vaccines. These methods rely on either the whole viral unit in attenuated and inactivated states, or viral subunits like DNA and toxins in natural, recombinant or conjugated forms. In general, subunit vaccines are often more effective vaccine alternatives with lower cost of production. These subunits are comprised of single or multi-epitope peptides that can increase immunity against the specific virus following vaccination (Clem). However, this approach requires an initial epitope discovery to predict the B- and T-cell epitopes for immune system stimulation. Peptide libraries comprised of overlapping mimotopes - peptides mimicking epitopes - are very useful in simplifying epitope screening and evaluating the effectiveness of a vaccine. Synthetic peptides as vaccines are specifically advantageous because of their chemical stability, T-cell subset stimulation, easy quality control, and absence of toxic, infectious or oncogenic components (Riemer *et al.*).

Custom peptides synthesized by GenScript helped Xin and Cutler to patent peptide and conjugate vaccines for fungal infections: *US 9416173 B2, 2016.*

Drug Discovery and Development

The main mode of action for most therapeutics is modulating or correcting aberrant cell signaling pathways. Key molecules such as lipids, carbohydrates and nucleic acids play important roles in ligand-target interactions. However, peptides produced as fragments of proteins or stand-alone cytokines and hormones can be considered as effectors in most signal transduction pathways, and hence, ideal targets for drug development (Otvos & Wade). In spite of having this advantage and the success of pioneer drugs like insulin, until recently poor delivery methods and rapid digestion of peptides rendered them ineligible to compete with small molecules for drug development. The revived interest in peptides as ideal drug targets in recent years is the result of limitations of small molecule drugs, discovery of unique characteristics of peptides, and advancements in peptide-related technologies. Increased resistance to existing drugs, lack of target specificity and poor delivery of current therapeutics, emergence of new pathogens and discovery of new mechanisms of disease formation and drugs' side effects are limiting the application of non-peptide drugs. On the other hand, low toxicity, biocompatibility, high target specificity and cell-penetrating ability of peptides, derived from their natural selection over millions of years, provide peptides with ideal features as therapeutics. Lastly, and most importantly, improvements in peptide design, synthesis and analysis technologies have drastically helped to overcome the majority of pharmacodynamic weaknesses of peptides (Firer & Gellerman). Furthermore, these technologies have helped with the expedited discovery and validation of novel drug candidates through high-throughput peptide library screenings (Craik *et al.*).

Advantages of Peptides as Drugs

- Unique intrinsic properties
- Excellent pharmacological profile
- High specificity, safety, tolerability, and efficacy
- Low complexity production
- Affordable

GenScript's variety of high-quality **Peptide Libraries** offer powerful tools in drug discovery research.

As therapeutics, peptides can be used as drugs, biomarkers and diagnostic tools. As drugs, peptides are used either directly as modulatory agents in the form of agonists, antagonists, vaccines and antibodies or act as carriers of cytotoxic agents, radioisotopes and ribonucleotides. Homing peptides, like RGD and NGR, that target very specifically to various normal or diseased tissues, as well as cell penetrating peptides (CPPs) , function in the latter manner. On the other hand, as ligands or mediators of many biochemical pathways, peptides can serve as diagnostic tools and biomarkers in disease diagnosis and progression (Thundimadathil).

The design of therapeutic peptides is inspired by the naturally-occurring peptides. Bioactive peptides are produced in all taxonomical species and exert various biological roles inside or outside of an organism. For example, toxic venom peptides that interfere with the neural transmission of the prey have successfully been used for the treatment of pain, cardiovascular diseases, diabetes and cancer (Lewis & Garcia). Natriuretic peptides are another class of peptides expressed in tissues like brain and heart, which are considered as both biomarkers and therapeutics (Ichiki & Burnett; Meems & Burnett). Natural peptide drugs, however, are limited by their extensive renal clearance, nonspecific tissue uptake and sensitivity to enzymatic degradation. Advancements in bioinformatics and peptide manufacturing has helped to overcome such limitations. As a result, systematic analysis of bioactive peptides and their pharmacological promise is now the foundation of the young field of "Peptidomics". The massive datasets generated by this analysis is used for specific AA modifications to increase the delivery and efficacy of peptide-based drugs. In addition, a multi-target approach in which a peptide drug can stimulate more than one biological process can be employed in order to obtain a more efficacious and personalized treatment (Fosgerau & Hoffman). Promising and successful clinical data on the application of peptides in drug discovery and development has already initiated discussions on the concept of "Peptides for Drugs" rather than "Peptides to Drugs" (Uhlig *et al*.).

Naturally Occurring Peptides as Therapeutics

Strengths	Opportunities
Good efficacy, safety and tolerability	Discovery of new peptides
High selectivity and potency	Focused libraries and optimized sequences
Predictable metabolism	Formulation development
Shorter time to market	Multifunctional peptides and conjugates
Low attrition rates	Alternative delivery routes
Standard synthetic protocol	

Adapted from Fosgerau & Hoffman 2015.

Custom peptides synthesized by GenScript helped Baud *et al.* develop a novel peptide-based screening for the identification of pluripotency markers:

Multiplex High-Throughput Targeted Proteomic Assay To Identify Induced Pluripotent Stem Cells. Analytical Chemistry, 2017.

Immunotherapy

The foundation of immunotherapy is based on modulation or employment of key players of the immune system for disease management and treatment. These components range from immune cells, such as B and T cells, to immune checkpoint and regulatory molecules, such as PD-1 and cytokines. Regardless of the point of intervention, different types of immunotherapy aim at stimulating, augmenting or suppressing an immune response. Currently, main categories of immunotherapy include therapeutic antibodies, vaccination, immune system modulators, immune cell therapy, and immune checkpoint modulators. Application of immunotherapy in a variety of tumors has drastically changed the landscape of cancer treatment. However, immunotherapies are also being adopted for the treatment of Alzheimer's disease, allergic rhinitis, and asthma (Asaria *et al*.; Delrieu J. *et al*).

Peptides, specifically, are being increasingly utilized for either improving existing forms of immunotherapy or developing novel immune-based drug platforms. This renewed interest in the application of peptides in immunotherapy is due to several factors. First, improvements in the synthesis of more stable peptides through cyclization or incorporation of D-amino acids have overcome the initial concern over peptides low stability. Second, peptide-based immunotherapies are recognized to be associated with less side effects as a result of their decreased immunogenicity and mode of action (Moldaver & Larché). Furthermore, antigen recognition and epitope identification, which are the bases of most types of immunotherapies, fall more specifically under the unique features of peptides and their delivery formats.

For example, modulating or engineering T-cells, which are the most widely-employed immune component in immunotherapy, greatly benefits from peptides. T cell epitopes are short peptide sequences, ranging from 8-11 AAs on MHC-I and 15-24 AAs on MHC-II complexes. Precise and quick identification of these epitopes is powered by high-throughput peptide delivery formats, such as libraries, that span hundreds of peptide combinations simultaneously. Following this peptide-based identification of most-stimulatory epitope, peptide synthesis technologies enable the development of a peptide-based immunotherapy with higher stability and tissue targeting. The synthetic peptide then is either used directly (in peptide vaccines), or indirectly as a delivery method (in CPP or homing peptides) or a component of another therapeutic molecule for specific antigen detection (in antibody-peptide fusion drugs) (AlDeghaither *et al.*). Similarly, development of chimeric antigen receptor T-cell (CAR-T) immunotherapy

or engineering designer T-cell receptors (TCR), for targeting tumor associated-antigens (TAAs) and neoantigens benefit from peptides. Encouraging results from clinical studies using peptide immunotherapy alone or in conjunction with other therapeutic modalities will continue to expand the design and application of peptides in modulating immune system.

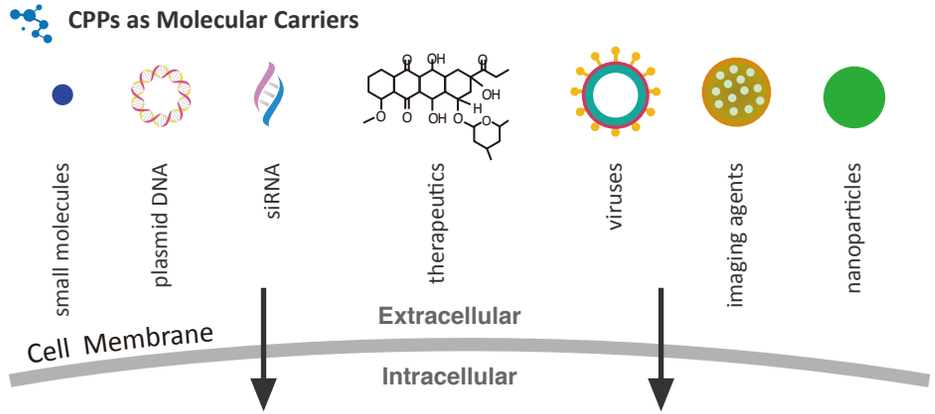
Custom peptide libraries synthesized by GenScript were used by Tittarelli *et al.* to design T cell receptors for targeting cancer antigens:

Changing the peptide specificity of a human T-cell receptor by directed evolution. Nature Communication; 2014.

Cell Penetration-Based Applications

In 1988, the purified trans-activator of transcription (TAT) protein from the HIV-1 virus was shown to be easily taken up by cells through a transduction domain (Frankel & Pabo). This was a great discovery given the known difficulty of peptides passage through the plasma membrane as a result of their molecular weight and hydrophobicity. Yet, it took more than a decade from this discovery of cell penetrating peptides (CPPs) to find their much-needed application in the delivery of hard-to-pass molecules into cells. CPPs are small and relatively non-toxic, short cationic and/or amphipathic sequences and many of them are derived from natural proteins or peptides. Contrary to microinjection and electroporation-based delivery methods of hydrophilic macromolecules, CPPs employ physiological mechanisms that maintain the integrity of cell membrane (Farkhani *et al*). In chimeric CPPs a combination of sequences are used where one sequence is specific to transmembrane passage through the cell membrane and the other through the nucleus (Zhang *et al.*).

Application of peptide libraries has facilitated the screening of a variety of CPPs (Ramsey & Flynn). These peptides can be used as direct therapeutic agents or linked to other non-cell permeable agents. In particular, conjugation of CPPs with nanoparticles is revolutionizing drug delivery systems, biosensors, and biological assays. Successful application of CPPs in biotechnology and medicine is dependent on the type of CPP and its concentration, transported cargo, as well as cell type. In cancer therapy, specifically, due to the lack of CPP's antigenicity, simple structure and diverse synthesis methods, application of peptide-based drug conjugates (PDCs) has not only decreased the off-target side effects and morbidity associated with chemotherapy, but also provide access to difficult-to-penetrate regions, such as brain (Gilad *et al.*; Zhang *et al.*).



Anti-Microorganisms Applications

Among the rapidly advancing applications of peptides is their use in targeting a variety of pathogenic microorganisms: bacteria, fungi, yeast and viruses. This phenomenon is the result of tolerance to existing drugs, lack of effective therapies or emerging pathogens. For example, anti-microbial peptides (AMPs), which comprise the majority of this category of peptides, are the alternative solution to the increasing resistance to existing antibiotics (Di Luca *et al.*). In the case of anti-fungal peptides, similar needs have led to the development of multivalent peptides, which are developed through the assembly of monomeric peptides around a core molecule (Lakshminarayanan *et al.*). Antiviral peptides against viruses such as HIV, Dengue, and influenza have also been developed. The use of peptides as antiviral agents are highly advantageous considering that they can also fight the bacterial infection that usually ensues a viral infection (Skalickova *et al.*).

This category of peptides are either isolated and purified from their natural source or synthesized and modified based on natural templates. The natural bioactivity of peptides residing outside an organism for defense purposes has evolved over billions of years and is the foundation of AMPs (Epand & Vogel). Venom of a variety of insects and reptiles or chemicals in amphibian skins are used against other microorganisms (Uhlig *et al.*; Li *et al.*). Similarly, in plants a variety of these compounds are ubiquitously produced in stems, roots, flowers, seeds, and leaves as part of the plant's immune response (Salas). AMPs can also be incorporated in biofilms. The thin layer of microorganisms called biofilms colonize on various natural (like intestine and skin) or synthetic (such as implants and dental plaques) surfaces to fight against wound infections, chronic disease or medical device-related infections (Strempel *et al.*). Specifically, adoption of the multivalency strategy is facilitating the clinical application of AMPs by enhancing their stability, efficiency, maintenance of activity and lowering toxicity (Liu *et al.*).

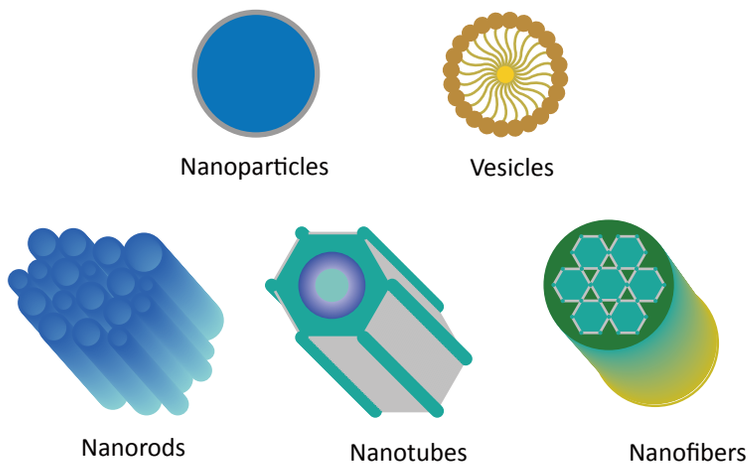
Custom peptides synthesized by GenScript helped Taute *et al.* discover an intracellular target for an AMP:

Investigation into the mechanism of action of the antimicrobial peptides Os and Os-C derived from a tick defensing. Peptide, 2015.

Tissue Engineering and Regenerative Medicine

The two-way communication between cells and the surrounding extracellular matrix (ECM) microenvironment is key in tissue development, maintenance and regeneration. Disturbances in this interaction can lead to organ failure and disease. To reverse or amend such failure, efforts in the area of regenerative medicine and tissue engineering in the last two decades have focused on the use of biomaterials such as peptides to reconstruct the ECM and to provide a scaffold for stem cells growth (Pugliese & Gelain). The most prominent examples are the self-assembling peptide hydrogels (SAPHs), which are comprised of short, amphipathic AA sequences. Upon exposure to the right stimuli, SAPHs form hydrogels and can easily be modified to control mechano-structural properties in scaffolds and carriers. SAPHs-based nanofibers, nanorods, nanotubes, nanovesicles, and nanoparticles have broad applications in biology, tissue engineering, medicine, and drug delivery (Tang *et al*.; Vauthey *et al.*). With the successful testing of peptidic biomaterials *in vivo*, their potential as biomimetic nanostructured scaffolds in clinical settings is gradually becoming a reality.

Self-Assembling Peptides



Custom peptides synthesized by GenScript helped Kaltofen *et al*. test the functionality of a uniquely designed self-assembling peptide.

Computational de novo design of a self-assembling peptide with predefined structure. J Mol Biol., 2015.

Cosmetics

Over the course of human history, man's desire to reverse the process of aging has led to the discovery and development of a variety of compounds and devices for masking, reducing or correcting signs of aging. One of the recent advancements on this front have been the application of peptides as active ingredients in cosmetic products. These peptides target intrinsic cellular processes of aging, such as the generation of reactive oxygen species (ROS), loss of ECM proteins, decrease in cutaneous blood flow, and loss of cells and their function. Currently, there are three main categories of cosmetic peptides: (a) signal peptides to increase the synthesis or inhibit the breakdown of collagens, (b) neurotransmitter-affecting peptides to mimic the effects of botulinum, and (c) carrier peptides for delivering trace elements required for enzymatic processes (Lupo & Cole). The broad acceptance of peptides as natural molecules, their stability and well-defined actions as well as the change in demographics and people's choice guarantees a promising future for this application of peptides.

Food Industry

The newest application of peptides in food and agriculture industry is growing from infancy. This growth is largely due to the increasing rate of human population, vulnerability of agriculture to disease and enhanced awareness about the health and environmental impact of chemical additives and pesticides (Keymanesh *et al.*). Peptide-based food additives are used as taste-substitutes (like Aspartame), preservatives (like Lactoferricin), antimicrobial (like Nisin) or biosurfactants in manufacturing process (Wang *et al*.). Current attempts to reduce the production cost of peptides compared to traditional chemicals will help with future application of peptides in food industry.

Chapter Three

Synthesis of Peptides

Custom peptide synthesis allows for the replication of naturally occurring peptides and the development of novel peptides to address specific needs or optimize specific processes. Depending on the scale of production, desired modifications, and application of custom peptides, the method of synthesis can vary. In this section, we will review both common and modern strategies of peptide synthesis as well as their advantages and limitations. We will then discuss purification and modification of peptides with a summary of important considerations in designing custom peptides.

Major Synthesis Strategies

A. Chemical Synthesis

A.1. Liquid and Solid Phase Synthesis

There are two main methods for chemically synthesizing peptides: liquid-phase synthesis (LPS) and solid-phase synthesis (SPS). Early attempts in forming peptide bonds in laboratory date back to 100 years ago, but due to the complex nature of this process, the commercial synthesis of peptides such as insulin came along decades later. Today the classical method of LPS coexists with the more mainstreamed method of SPS, where due to technological advancements each have evolved into easier and more reliable processes.

The chemical process of peptide synthesis in both LPS and SPS is developed as a step-wise and cyclic procedure to ensure proper incorporation of AAs to the growing peptide chain. Peptide synthesis in vitro occurs in a C- to N-terminal direction, which is opposite to the direction of protein synthesis in biological systems *in vivo*. Synthesis is based on the formation of a peptide bond between two AAs in which the carboxyl group of one AA is coupled to the amino group of another AA. This process is repeated as long as all AAs in the peptide chain are assembled and the desired peptide sequence is obtained.

LPS and SPS share many procedural overlaps with only a few differences. The side chains of all AAs in both methods are capped with specific “permanent” groups, which can withstand continuous chemical treatment throughout the cyclical phases of synthesis, and are cleaved just prior to the purification of nascent peptide chain. Additionally, the N-terminal of each incoming AA in both methods are protected with “temporary” groups, which are removed in each cycle to allow for the incorporation of the next AA to the chain. These groups prevent non-specific reactions during synthesis that would lead to changes in length or branching of the peptide chain. Protecting groups are removed with an acid or base through a process called “deprotection” or “decoupling”. This procedure usually results in the production of cations with the potential to alkylate the functional groups on the peptide chain. Therefore, scavengers such as water, anisol or thiol derivatives are added during deprotection to block free reactive species.

Two N-terminal protecting groups are commonly used: 9-fluorenylmethoxycarbonyl (Fmoc) and tert-butoxycarbonyl (Boc). Both of these groups contain a carbamate group that can readily release CO2 for an irreversible cleavage when necessary. Specific application of each of these protecting groups, however, is based on their unique characteristics. For example, since Fmoc can be removed with a mild base such as piperidine, it is commonly used in commercial settings. On the other hand, since removal of Boc requires a moderately strong acid such as trifluoracetic acid (TFA), it is used where base-sensitive peptides or aggregation reduction is called for.

The key difference between LPS and SPS lies at the initiation step. In SPS, the C-terminal of the first AA is coupled to an activated solid support, which protects the C-terminal from participating in unwanted side reactions. The solid support is usually a synthetic polymer carrying a reactive group like –OH to react with the carboxyl group of the first AA for a covalent linkage. In LPS, on the other hand, the C-terminal of the first AA has to be protected with a cap or permanent protecting group since the reaction is taking place in liquid phase.

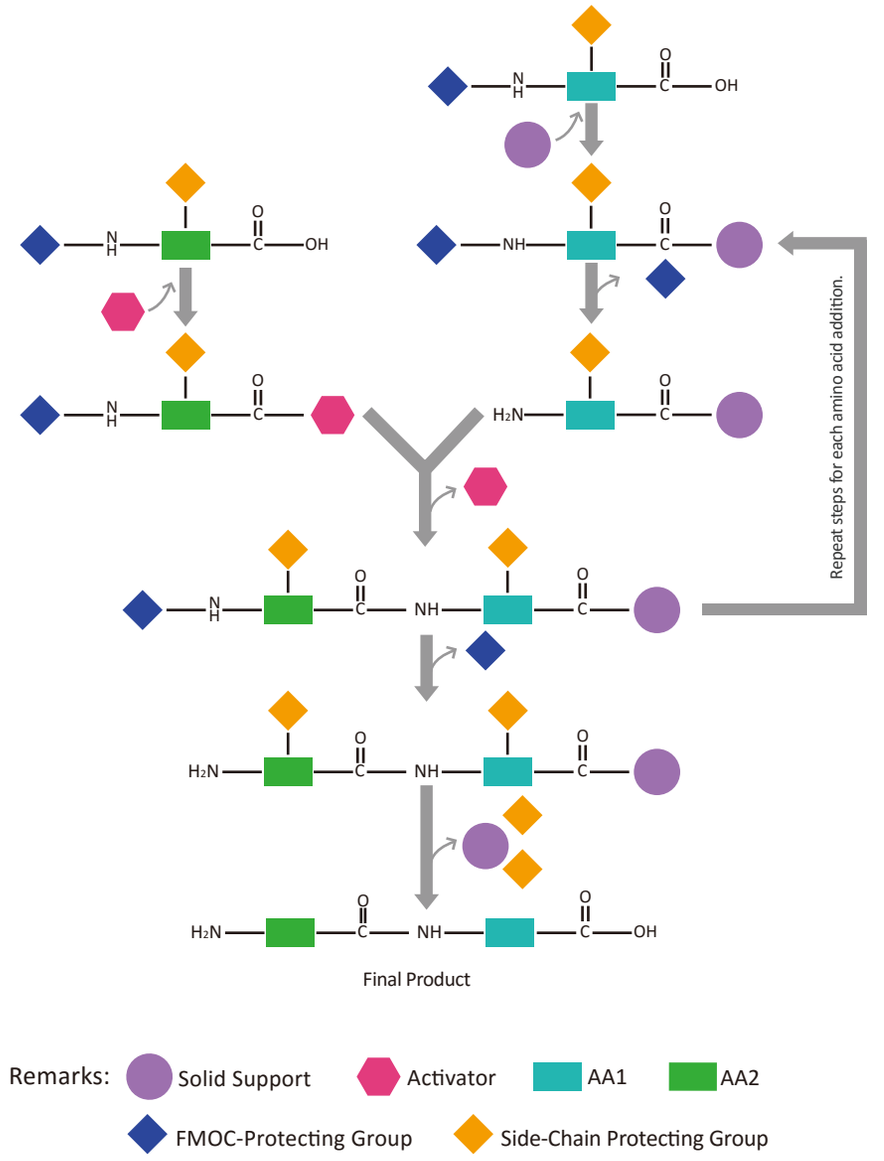
In addition to differences in AA protection, applications of LPS and SPS methods are slightly different. LPS is best for the commercial synthesis of peptides with less than 8 AAs. Whereas the manual removal of product after each step renders this method slow and laborious, continuous purification after each step, allows easy detection of side reactions. Furthermore, it allows ligation of separate peptides to generate larger peptides. The SPS method, on the other hand, is preferred for manual or automated synthesis of peptides longer than 8 AA residues.

A.2. Microwave-Assisted Technology

Common methods of peptide synthesis, though effective in the synthesis of many peptide sequences, are associated with challenges that are inherent to their properties. A short list of such issues includes lower success rates with difficult peptides, increased chance for intra- and/or intermolecular aggregation, increased likelihood for secondary structure formation and steric hindrance of protecting groups which can generate premature sequence termination. To overcome these challenges, an alternative approach utilizing the microwave energy was adopted in peptide manufacturing. This technology, which is generally combined with SPS, not only resolves the limitations of common synthesis methods, but also improves other features of the synthesis process and the synthetic peptide product itself.

The fundamental of this technology is the source of heating, which is generated through the agitation of polar molecules or ions under the effect of an oscillating electric or magnetic field (Rizzolo *et al.*). Generated heat is then used throughout the process of peptide synthesis (i.e. coupling and deprotection reactions), as well as the post-synthesis cleavage step for removing side chain-protecting groups and the final peptide product from the resin. Given the presence of many polar and ionic species in the reaction (i.e. bases for deprotection, polar solvents, polar/ionic activators, and the

Step-Wise and Cyclical Process of Peptide Synthesis



terminal amine group), the resulting increase in temperature helps break up chain aggregation and allows for easier access to the growing end of the peptide chain. This in turn leads to increased peptide purity, higher yield, greater reproducibility, and the ability to synthesize long and difficult peptides which might form β-sheet structures or have sterically difficult couplings (Palasek *et al.*; Collins *et al.*). Moreover, side chain manipulations, C-terminal peptide modification, and the synthesis of a variety of peptidomimetics can greatly benefit from the microwave-assisted technology.

Currently, disadvantages of this technology are limited to dehydration and racemerization that may occur as a result of Cysteine and Histidine coupling. However, due to superior advantages of this technology for the synthesis of high quality peptides in a short period of time, concerted efforts are already underway to rectify these issues. The versatile nature of microwave-assisted technology and the rapid advancements in associated methods is foreseen to enable the synthesis of more complex peptides and peptide conjugates for diverse biomedical applications.

A.3. Ligation Technology

Synthesizing long peptides can be accomplished by ligating smaller peptide chains through chemical and enzyme-mediated technologies. In the chemical approach, peptide fragments can be linked together through a procedure called "fragment condensation". In using this method, however, strategies against racemization and interfering byproducts have to be considered. Alternatively, the more recent method of "Native Chemical Ligation" (NCL), can be used in which a native bond is formed between the C-terminal thioester and the N-terminal Cysteine of two unprotected peptide segments. The reaction progresses after an initial chemoselective thiol-thioester exchange followed by an irreversible intramolecular S,N-acyl shift to form the desired peptide bond (Dawson *et al*.). This procedure has advantages such as high yield, high chemoselectivity, absence of C-terminal racemization and use of a neutral aqueous media which is ideal for the solubility of unprotected peptide segments. Conversely, its limitations stemming from the incompatibility of the thioester functionality with automated Fmoc SPS and low abundance of Cysteine in naturally occurring peptides has led to the development of newer methods, such as Cysteine-free NCL, α-ketoacid-hydroxylamine and Serine/Threonine ligation strategies (Noisier & Albericio).

Enzyme-mediated ligation technologies are the latest development in this area. These methods which are based on the existence of natural peptide ligases provide great tools for labelling, efficient and selective N-terminus coupling and macrocyclization of peptides. Peptide ligases such as sortase, butelase, trypsiligase, subtiligase, peptiligase and omniligase provide unique ligation opportunities based on their site specificity and substrate scope (Schmidt *et al*.). Enzyme-mediated technologies can be used alone or in conjunction with chemical ligation methods to achieve higher level of complexity and precision.

Comparison of Major Peptide Synthesis Methods

Strategy	Specifications
Liquid Phase	• Best for peptides with < 8 AA in large quantities • Generates only one product • Easy and inexpensive scale-up • Formation of side reactions easily detected • Suitable for ligation technology • Difficult purification • Not highly automated • Long production cycle • Requires an additional step in protecting the C-terminal of the first AA
Solid Phase	• Best for long peptides in small quantities • Easy purification • Highly automated • Short production cycle • Generates a mixture of products • Expensive scale-up • Requires two extra steps of linkage and cleavage
Microwave-Assisted	• Faster reaction times • Better peptide yield and purity • More effective for difficult-to-synthesize peptides • Better reproducibility • Increased chance of dehydration or racemerization with some AAs
Recombinant	• Best for biologically active or immunogenic peptides in large scale • Flexibility in introducing unnatural AAs • More complicated solubility

B. Recombinant Technology

In recent years, genetic engineering and recombinant protein technologies have found their way into peptide synthesis. Advantages of this method over peptide chemistry include the synthesis of peptides with higher complexity or length, biologically active conformation or higher immunogenicity. In addition, manipulation of expression host and the use of redundant and 4-base pair codons for introducing unnatural AAs into the peptide chain, provide more flexibility in peptide design (Young & Schultz). On the other hand, recombinant processes require careful design and higher quality assurance. For example, since most recombinant peptides need to be fused to a carrier molecule to increase solubility and avoid the formation of inclusion bodies, special consideration should be given during the purification stage to ensure proper peptide folding, activity and yield (Vriens *et al.*).

Modifications

The possibility of incorporating endless modifications into a peptide chain is among the top advantages of custom synthetic peptides. From mimicking natural post-translational modifications (PTM) for bioactivity to introducing enzymatic and fluorescent tags for site-specific activation and *in vitro* manipulations, custom modifications serve as useful tools. Peptide modifications can be sequence/residue specific or tailored towards one or both end terminals. Whereas some molecules can be directly linked to the peptide chain, addition of others requires the presence of other molecules called “linkers” or “spacers”. These are single, flexible molecules or a stretch of molecules that link the desired modification to the peptide chain. For example, for efficient N-terminal labeling with the fluorescein fluorophore, a seven-atom aminohexanoyl spacer (NH2-CH2-CH2-CH2-CH2-CH2-COOH) is inserted between fluorophore and N-terminal of the peptide. A thorough understanding of the peptide sequence and specific function of a desired modification is key in designing a custom modified peptide to ensure proper functionality of the synthetic peptide in downstream applications.

GenScript’s **FlexPeptide™ Peptide Synthesis Technology** is an advanced integrated approach for synthesizing the most difficult peptide sequences with the highest synthesis success rate in the industry.

Peptide Modifications and Their Application

Modification	Application	Example
C-terminal	• Mimicking natural protein structure • Charge balance	Amide, Acid, Aldehyde, Ester, AMC, OMe, Oet, TBzl, Cya, NHMe, NHEt, pNA, Tags
N-terminal	• Mimicking natural protein structure • Stabilization	Formylation, Acetylation, Fatty Acid, Benzoyl, Urea, Carbamate, Succinyl, Alkylamine, Sulfonamide, Boc, Mpa, Tags
Post-translational	• Application-specific cysteine modifications • Studying cell processes	Glycosylation, Methylation, Palmitoylation, Acylation, Myristoylation, Phosphorylation
Fluorescent Tags	• Binding and localization studies	FAM, Alexa Fluor, Cy3, Cy5, Abz, FITC, TAMRA
FRET / Quenching Tags	• Molecular interaction studies • Enzymatic assays	FAM, DANCYL, DABCYL, EDANS/DABCYL, FITC, MCA
Non-fluorescent Tags	• Molecular detection and isolation	Biotin, Flag, Myc, HA
Immunogenic Conjugates	• Enhancing immunogenicity for antibody production	BSA, HAS, OVA, KLH
Linkers / Spacers	• Enhancing stability and bioavailability	Ahx, Ttds, PEG, β-Ala
Unnatural AAs	• Induction of secondary structure • Enhancing stability, selectivity and activity	β-AAs, γ-AAs, D-AAs
Cyclization	• Peptide optimization • Enhancing stability, selectivity and activity	Side to side, Head to tail, Head to side, Side to tail
Stable Isotope Labels	• Molecular detection and quantification • NMR Spectroscopy	^{15}N, ^{13}C, ^{2}H

Purification

In general, peptide impurities are the result of the cyclic nature of peptide synthesis, multiple protection/decoupling steps, or incomplete synthesis. Since presence of these impurities affects downstream application of peptides, a final purification step is usually included in the process of synthesis. The choice between different purification methods depends on the size, charge and hydrophobicity of peptides. Whereas size-exclusion chromatography, partition chromatography, and ion-exchange chromatography offer reliable purification of synthetic peptides, reverse-phase high performance liquid chromatography (RP-HPLC) is considered the most widely used and versatile method. In the process of purification, peptides bind to the HPLC column through hydrophobic interactions and are eluted by increasing the hydrophobicity of the eluent using a variety of buffers such as phosphoric acid, TFA/TEA (trimethylamine), heptafluorobutyric acid, HCl, formic acid, sodium/ammonium acetate, and sodium/potassium phosphate. This method separates target peptides from deletion or truncated sequences, isomers, and peptide products containing side-chain reactions. Alternatively, for the purification of very long peptides or large quantities of peptides, Ion exchange HPLC is used.

At the end, purity of synthetic peptide is calculated based on the amount of the correct peptide relative to the total amount of all analytes in a synthetic prep with absorbance at 214 nm. The percentage of purity can range from low in crude preps to very pure at 98%, and can be customized based on downstream applications. Whereas a peptide with the highest purity is advantageous for all applications, cost considerations dictate the range of acceptable purity for obtaining the most reliable results.

Peptide Purity Guidelines

> 70%

Best for Immunological Applications

- Immunological assays
- Polyclonal antibody production
- Affinity purification

> 85%

Best for Biochemical Applications

- NMR
- Epitope mapping
- Cell-based assays (bioassays)

> 95%

Best for Purity-Sensitive Applications

- Structure-activity relationship (SAR) studies
- Quantitative assays
- *In vitro* bioassays

> 98%

Best for Industrial Applications

- Crystallography
- cGMP peptides for drug studies
- Clinical trials

Product Identity and Quality Control

The final step in the production of a synthetic peptide is checking the identity and quality of the synthesized product. In general two categories of testing are performed:

A) Quantification testing: using RP-HPLC and MS components of the synthetic prep are identified and quantified. The percentage of various components such as target and total peptide content, water and organic solvents are measured according to their specific absorption wavelength. If detailed information about the exact AA composition of the synthetic peptide is needed, an amino acid analysis (AAA) is performed. To determine trace amounts of water content in the peptide sample the Karl Fischer colorimetric titration can also be carried out.

B) Toxicity testing: during the process of synthesis, some non-peptide molecules, which are toxic to cells or interfere with downstream applications, may be generated. Some molecules like TFA are removed using ion chromatography whereas endotoxic liposaccharides can be cleared with chromogenic T lysate or limulus amebocyte lysate testing.

GenScript's **Total Quality Management** platform ensures that each custom peptide is multiple-checked in QC & QA to guarantee the delivery of high-quality peptides.

Key Peptide-Related Terminologies

Terminology	Definition	Determined by
Net Peptide Content	• Actual weight of the peptide component in a sample • 50-80% of the total peptide weight	• Quantitative amino acid analysis or UV spectrophotometry • Theoretical calculation = MW of peptide/MW of peptide + (number of counter-ions x MW of counter-ion)
Total/Gross Peptide Content	• Actual contents in a sample • Includes peptide component, water, absorbed solvents, counter ions and salts	• MS
Peptide Purity	• Percentage of the target peptide sequence in the peptide component of a sample	• Ratio of peak area of target peptide in relation to all detected peak areas in MS analysis

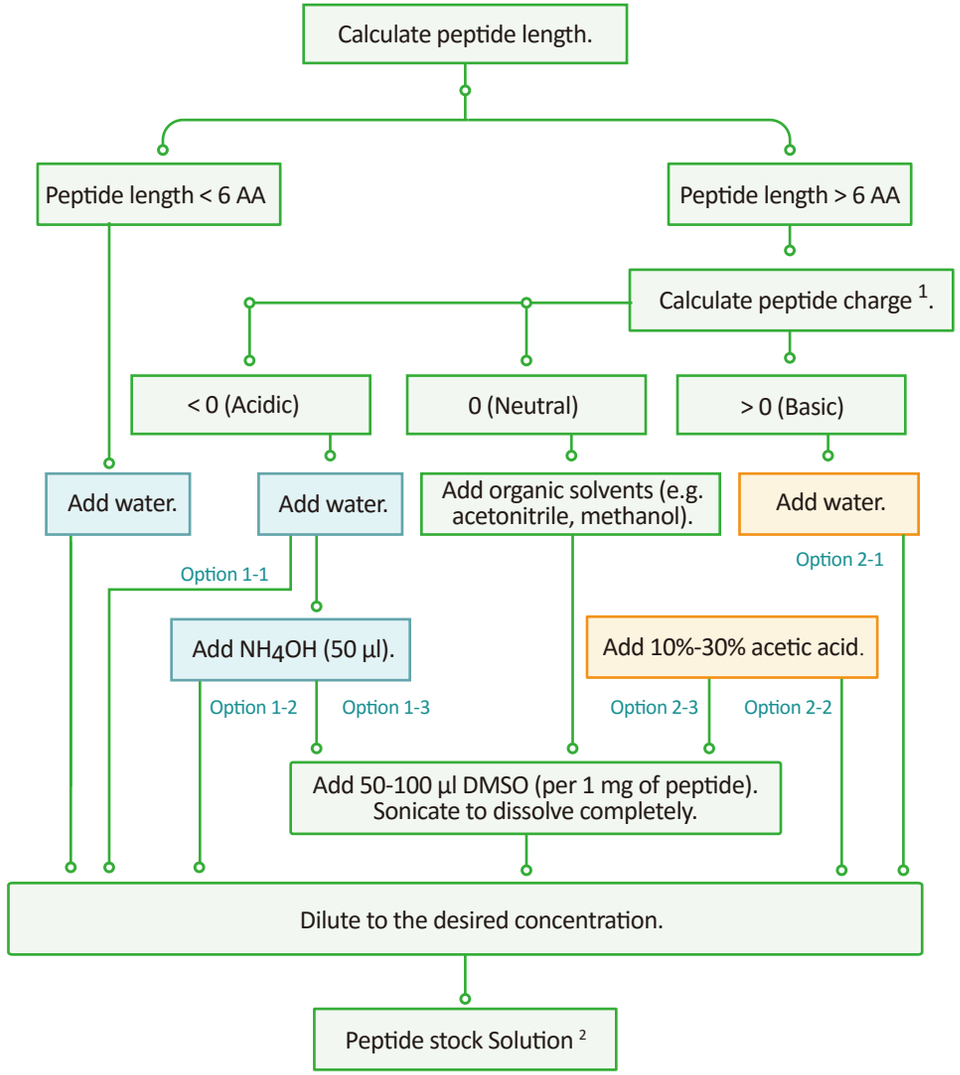
Reconstitution, Storage and Handling

Custom peptides synthesized by service companies for end users are typically package in lyophilized form. To ensure long-term viability, the peptide product is aliquoted in small microfuge tubes and shipped at room temperature. Once the product is in the hands of the customer, extreme care needs to be given to proper storage and reconstitution of the lyophilized peptide.

It is recommended to refrigerate lyophilized peptides and store them away from bright light. For short-term storage ranging from one week to two months, peptides may be stored at 4°C. However, long-term storage requires keeping the peptide at -20°C. Even though these conditions keep most peptides stable for several years, the long term stability of peptides is always at risk. Therefore, careful planning of experiments, minimizing vial openings and tightly closing the cap will help in reducing the risk.

Prior to starting an experiment, stored tubes need to be acclimated to room temperature to avoid condensation of atmospheric water. Most peptides can easily be reconstituted in water. The general rule of diluting stock solutions is to start with adding water and then try other solvents if precipitation occurs. The ideal solvent can be selected based on the overall charge of the peptide chain. Leftovers of the reconstituted peptide should be immediately kept in small aliquots at -20°C to avoid condensation, microbial contamination or oxidation of residues such as Cysteine, Methionine and Tryptophan.

Guideline for Reconstituting Synthetic Peptides



[1] Assign a value of -1 to acidic residues, i.e. Asp, Glu, and the C-terminal -COOH. Assign a value of +1 to basic residues, i.e. Arg, Lys, His, and the N-terminal -NH2. Calculate the overall charge of the entire peptide.

2 It is recommended that the concentration of the stock solution be around 1-2 mg of peptide per 1 mL of solution. This is dilute enough so that relatively small volumes (< 100 µL) of peptide can be used in an assay; minimizing the effect of solvents initially used for solubilization.

Delivery Formats

In general, synthesized peptides are delivered in lyophilized powder form in a vial. While this delivery method is still the most widely used delivery format, emergence of new application of peptides and the need to answer biological questions from a different perspective has led to the development of novel delivery formats. Custom synthetic peptides can now be deposited in multi-well plates as libraries or printed on membranes as microarrays. Having a clear understanding of the downstream application is key to identifying the best delivery format; ensuring cost effectiveness and relevant, reliable results.

GenScript's **ArgonShield™ Packing Service** eliminates the experimental variation caused by peptide oxidization and deliquescence of your high-quality custom peptides.

Custom Peptide Delivery Formats

Format	Type	Scale	Application
Single Vials	Standard	Milligram	All peptide applications
	Large	>1mg to Kg	Biological additives in healthcare products or therapeutics
Library	Standard	Milligram	Proteomics, Bioassays, Epitope mapping Immunotherapy, Design and development of therapeutics and vaccines
	Micro	Micromolar	Targeted proteomics applications, Preliminary peptide screening
Microarray	Standard	Nanomolar	Antibody epitope mapping, Molecular interactions studies, Phosphorylation studies, Binding and functional assay studies, B-cell epitope mapping

Considerations in Peptide Design

Functionality and application of a synthetic peptide is directly linked to its design. Given that there is more to peptide design than writing the name of AA residues in a sequence, special attention needs to be given to chemical properties, placement and modification of each residue within the chain to ensure the successful synthesis of a custom peptide. Considering the following guidelines will help in obtaining a functional peptide:

General Guidelines

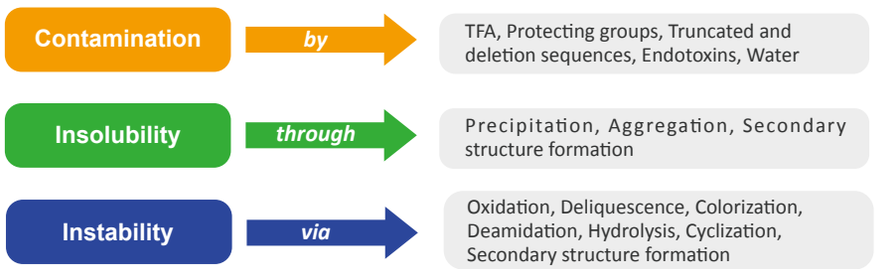
- Be familiar with the chemical properties of each AA and modifications.
- Know the general processes of peptide synthesis, purification and handling.
- Have a clear idea of the specific application of your synthetic peptide.

Specific Guidelines

- The longer the peptide chain, the higher the chance of aggregation, peptide truncation, purification difficulties and low yield.
- Higher number of hydrophilic AAs leads to increased water content in the final product.
- Peptides with zero or polar charge tend to be insoluble. Amino acids such as Tryptophan, Valine, Isoleucine, and Phenylalanine decrease solubility whereas AAs like Lysine, Histidine, Arginine, Asparagine, Glutamic Acid, Serine, and Threonine increase solubility. Despite being non-polar and interrupting the β sheet, Proline helps with peptide solubility.
- To ensure the solubility of your peptide, keep the hydrophobic AA content of your peptide chain below 50%, and include one positively charged residue for every 5 AAs.
- Addition of a spacer can affect peptide solubility. For example, a spacer like PEG can increase peptide solubility in general. On the other hand, fatty acids such as palmitic acid or myristic acid will decrease peptide solubility, but increase the transmembrane effect.
- Peptides containing hydrophobic AAs are more difficult to synthesize and result in reduced yield.
- Capping N- and C-terminals will make the synthetic peptide resemble the native peptide. The N-terminal can be capped with an acetyl group and the C-terminal with an amide group.
- Avoid long strings of Valine or Isoleucine since they may interfere with peptide chain extension during synthesis.
- Presence of certain oxidation-prone AAs like Cysteine, Methionine and Tryptophan can interfere with peptide cleavage and purification. Consider replacing Cysteine with Serine and Methionine with Norleucine.

- Avoid placing Glutamine at the N-terminal because it is unstable and will turn into cyclic pyro-glutamic acid. If this is not possible, consider acetylating the N-terminal.
- Avoid placing Asparagine at either terminal; especially the N-terminal, which can interfere with the removal of the protecting group.
- The presence of consecutive Serine residues in a peptide chain may lead to lower purity and higher deletion products as a result of isomerization.
- Since Asparagine is prone to hydrolysis and can lead to peptide cleavage in acidic conditions, avoid Asparagine-Serine, Asparagine-Proline and Asparagine-Glycine pairs.
- Remember that placing a tag at the N-terminal vs C-terminal makes the synthesis more straightforward.
- When adding tags, pay attention to the functionality of the peptide chain regarding the sequences close to its N or C-terminals.
- When adding a tag to your peptide chain, include a spacer between the peptide and the tag molecule. This will reduce the chance of the tag affecting peptide folding and binding to receptors. However, if the purpose of the tag labeling is to quantify fluorescence transfer between different structures, spacers should not be introduced.
- When incorporating phospho groups, place the phosphorylated residue no more than 10 residues away from the N-terminal to increase coupling efficiency.
- To generate antibodies using peptides, it is best to (a) have a hydrophilic peptide with the least number of Cysteine residues to minimize undesired disulfide bond formation, (b) avoid Arginine-Glycine- Asparagine (RGD) motif, Helix-Like-Helix (HLH) motif, GTP binding site, Src homology 2 domains or any functional and highly conserved motifs prone to conformational change to circumvent cross reactivity.
- Include an anti-oxidation agent like Dithiothreitol (DTT) in the reconstitution buffer when handling Cysteine-rich peptides.

Hidden Problems in the Synthesis and Application of Peptides



Chapter Four

Future of Peptides

The historic journey of peptides as essential components of bacterial, plant and mammalian cells across billions of years has evolved and expanded both their form and function. It is only in recent times, however, when the discovery of their rich universe provided us with a plethora of opportunities for exploring and improving human life. From applications in basic science research to therapeutics, the presence of peptides in our lives has greatly flourished and is anticipated to thrive even more in the future.

Our current knowledge of the chemical and structural diversity of peptides along with the rapid advancement of technology continues to further expand the boundaries of peptide design, synthesis and application. Discovery of new natural peptides combined with the power of bioinformatics and computer simulations will guide the rational design of novel peptides. Such information is already contributing to the burgeoning experimental studies on the use of synthetic peptides as protein mimics for modulating intermolecular interactions (Groß *et al*.). Advances in modern formulation and synthesis technologies will provide solutions for overcoming peptide limitations, lowering cost, higher stability and extended half-life. Furthermore, expanding and fine-tuning existing peptide delivery formats and modifications tailored towards emerging biomedical applications will ensue such advancements.

With the arrival of peptides golden era, widespread applications in science discovery, translational medicine and agriculture are fast growing. One specific umbrella of applications will reap the most benefit from this era; and that is medicinal therapeutics, diagnostics and theranostics. The appealing features of peptides for applications in medicine has for long been overshadowed by their limitations in delivery, stability and membrane permeability. However, new advancements in conjugating peptides to a variety of biologics and carrier molecules as well as clever peptide designs can circumvent such limitations (Bhardwaj *et al*.). Novel peptide-based immunotherapies, antibiotics, vaccines, and delivery methods as well as self-assembling peptides are foreseen to revolutionize all areas of medicine (Fosgerau & Hoffman; Pugliese & Gelain; Edwards-Gayle & Hamley). All we need to do now is to continue our creative use of peptides and imagine the endless possibilities they offer.

Chapter Five

Resources on Peptides

Peptide-Related Tools

To help facilitate the design and application of your custom synthetic peptides, GenScript has developed a comprehensive set of powerful tools. The following chapter familiarizes you with the scope of each tool before you are ready to use them online through our secure and encrypted system:

https://www.genscript.com/peptide_technical_resources.html

+ Peptide Chemical Formula & Molecular Weight Calculator

Calculates the chemical formula and MW of your peptide of interest. Simply enter the sequence data, modifications or location of disulfide bridges to obtain the desired information instantly.

+ Peptide Property Calculator

Calculates chemical formula, MW, hydrophilicity and isoelectric point of your peptide sequence, and suggests the best solvent. Enter sequence data, modifications and disulfide bridges to obtain a comprehensive list of properties for your peptide.

+ Antigen Prediction Tool (OptimumAntigen™)

Suggests three optimized peptide sequences as best antigens for generating a custom antibody. This tool uses a comprehensive peptide antigen database and selection criteria for optimizing the likelihood of surface exposure and antigenicity in a sequence yielding an ELISA titer of >1:64,000.

+ Peptide Library Design Tools

Familiarizes you with the variety of high-quality peptide libraries GenScript offers and helps you choose and design the right library for your application.

- Overlapping Peptide Library
- Alanine Scanning Library
- Truncation Library
- Positional Scanning Library
- Random Library
- Scrambled Library
- T Cell Truncated Library

Troubleshooting Guide

To help solve your peptide-related problems, use the following guide in conjunction with our Frequently Asked Questions page at our Peptide Technical Resources: ***https://www.genscript.com/faq_for_peptide.html.*** Our Ph.D.-level scientists are also available to answer your questions 24/7.

+ Problems with Quantity & Quality

Q. I ordered X grams of peptide, but the total amount of the peptide I received is less. What happened to the rest of my peptide?

A. This is due to the difference between net peptide content and total peptide content (i.e. what you ordered). The peptide shipped to you usually contains not only your desired peptide, but also other substances including water, absorbed solvents, counter ions, and salts. The total peptide content refers to the weight of this mixture. Net peptide weight indicates the actual weight of the peptide component of your sample and is usually 50-80% of the total/gross peptide weight.

Q. I used my reconstituted peptide successfully once, but it seems to have lost its activity after that. What is going on?

A. Peptides are bioactive molecules and as such they are subjected to loss of integrity and activity as a result of oxidation, deliquescence, colorization, deamidation, hydrolysis, cyclization, secondary structure formation and contamination. Make sure you follow all recommendations listed on page 32 for storing and handling your peptide.

Q. I can't completely dissolve my lyophilized peptide. What should I do?

A. The solubility of a given peptide depends on its AA sequence and modifications. Follow the guidelines on page 33 and consider the following tips for complete reconstitution of your peptide:

- Sonicate your peptide to increase solubility;
- Add 10% acetic acid to your solvent to help dissolve basic peptides;
- Add 10% ammonium bicarbonate to your solvent to help dissolve acidic peptides;
- For peptides with extremely low solubility try first adding organic solvents (DMSO, isopropanol, methanol, or acetonitrile). Once the peptides are completely dissolved, water may be gradually added until the desired concentration is obtained.

+ Problems with Application

Q. I ordered a peptide with 70% purity for my crystallography studies and it doesn't work. Why?

A. Peptide purity requirements depend on your specific application. The higher the purity of a peptide, the lower the amount of undesired contents and hence, interference with your experimental results. For sensitive applications like crystallography, you need to have a peptide purity of 95%. Make sure to check the peptide purity guidelines listed on page 30 before synthesizing a peptide. Also make sure that counter ions such as TFA are removed from your peptide.

Q. I ordered a custom peptide for *in vivo* studies, but it seems like the peptide is not long lasting and doesn't reach the desired tissue. Why?

A. *In vivo* application of peptides require specific modifications to ensure longer half-life, increased solubility, decreased renal clearance and increased tissue penetrance. Adding a PEGylated group, conjugating your peptide to nanoparticles or choosing a cell penetrating peptide can help with all of the above-mentioned issues to obtain ideal results.

Q. I generated a custom antibody based on a synthetic peptide, but the antibody is not working. What did I do wrong?

A. When using a peptide as an antigen for generating custom antibodies, you need to consider several factors such as AA compositions, length, hydrophobicity, and secondary structures to ensure maximum immunogenicity and efficiency. In conjunction with guidelines presented on Considerations in Peptide Design (page 35) and our online Peptide Antigen Design Tool, consider the following:

- Keep the length of your peptide sequence in the range of 10-20 residues;
- Design your peptide antigens from sequences that are found on the surface of the native protein;
- Select continuous, linear epitopes;
- Avoid common sequence motifs such as RGD, helix-loop-helix, GTP binding sites, and SH2 domains which may cause cross-reactivity.
- Avoid sequences associated with certain biological activities, such as autolytic cleavage, hormonal activity, and undesired post- translational modifications;
- Take advantage of conjugation chemistries (BSA or KLH) that best mimic epitope presentation and lead to higher immune response.

https://www.genscript.com/peptide-services.html

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Notes

GenScript's Peptide

Peptide Synthesis Services

- High-quality peptides synthesized at any length, purity, and quantity with more than 300 modification options
- Flexible delivery options for easy planning of experiments
  - ***Custom Peptide Synthesis Service:*** up to 200 AAs, mg to kg quantities, comprehensive modifications, all purity levels, delivered in 10-15 days
  - ***Express Peptide Synthesis Service:*** mg to kg quantities, over 30 modifications
    - ***Fast Peptide Synthesis:*** up to 20 AAs, all purity levels, delivered in 7-8 days
    - ***Rush Peptide Synthesis:*** 5-10 AAs, crude purity, delivered in 5 days

Peptide Library Services

- Systematic combination of a large number of peptides in different quantities and purity levels with comprehensive modifications offered in 7 varieties
- Diverse applications in immunotherapy, proteomics, and drug or vaccine discovery and development
  - ***Standard Peptide Library Service:*** milligram quantities
  - ***Micro-Scale Peptide Library Service:*** microgram quantities

Large-Scale Peptide Synthesis Services

- Multi-gram level custom peptide manufacturing for diverse applications, including **cosmetics** and **cGMP-level** drug and vaccine production
- Extensive reports on R &D, production and parameter quantification according to the nature of the project and customer's request

Services Portfolio

Peptoid Synthesis Service

- Synthesis of stable peptides with less susceptibility to degradation
- Ideal peptidomimetics for drug discovery

Click Peptide Synthesis Service

- Special chemistry for soluble β-amyloid synthesis
- Offers control over physiochemical properties and biological activities of the synthetic peptide

TFA Removal Service

- Recommended for cellular assays, API formulations and manufactured products
- Standard and Guaranteed service options

Solubility Testing Service

- Provides a full report on gross peptide concentration and best solvents for reconstituting peptides
- Takes the guesswork out of solubility options and saves the peptide for the desired application

Other Peptide-Specific Services

- Amino Acid Analysis
- Endotoxin Analysis
- pH Test
- Counter Ion Quantification Analysis
- Peptide Content Analysis
- Moisture Content Analysis