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Advances in Peptide Synthesis Technology and Applications

This article aims to analyze the development of peptide synthesis technologies, with a focused review on the origin, core principles, key technical aspects, current challenges, and future trends of SPPS. It is intended to provide comprehensive and in-depth technical reference for researchers and practitioners in the field.

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Beijing Dilun Biotechnology Co., Ltd.

Specializes in R&D and manufacturing of peptide synthesis equipment, providing comprehensive technical solutions and services.

Web: www.peptidescientific.com Email: duxiaoru@dilunbio.com

Peptides, as biologically active molecules formed by the covalent linkage of amino acids via peptide bonds, play a central regulatory role in vital processes such as cellular signaling, immune modulation, and neurotransmission. The precise synthesis of peptides not only provides a scientific basis for elucidating their structure–activity relationships but also serves as a key enabling technology for developing peptide-based drugs, novel biomaterials, and highly sensitive diagnostic reagents.

Since the early 20th century, when peptide synthesis research began, chemical synthesis—including both liquid-phase and solid-phase methods—has been the primary approach. After more than a century of technological evolution, solid-phase peptide synthesis (SPPS) has emerged as the dominant method in the field, owing to its unique advantages. It has greatly facilitated breakthrough advances in biomedicine, including targeted peptide therapeutics (e.g., GLP-1 analogs) and peptide vaccines.

This article aims to analyze the development of peptide synthesis technologies, with a focused review on the origin, core principles, key technical aspects, current challenges, and future trends of SPPS. It is intended to provide comprehensive and in-depth technical reference for researchers and practitioners in the field.

1. Liquid-Phase Peptide Synthesis (LPPS)

As the foundational method of chemical peptide synthesis, liquid-phase peptide synthesis (LPPS) originated in the 1920s. It involves two primary strategies: stepwise synthesis and fragment condensation, both of which are carried out in solution to assemble the peptide chain through consecutive condensation of amino acid residues.

In 1935, Harington and his team successfully synthesized the first biologically active tripeptide—glutathione—using this technique. In 1953, Vigneaud's group accomplished the total synthesis of oxytocin, for which he was awarded the Nobel Prize in Chemistry in 1955. This achievement not only demonstrated the feasibility of synthesizing complex peptides in solution but also laid the groundwork for subsequent studies on the synthesis and function of numerous bioactive peptides, significantly advancing the interdisciplinary development of peptide chemistry and biochemistry.

A defining characteristic of LPPS is that each condensation reaction requires isolation and purification of the intermediate peptide before it can be reintroduced into the liquid phase for the next round of coupling. Consequently, the method is associated with challenges such as cumbersome purification, complex operational procedures, and low automation. Nevertheless, LPPS offers several advantages, including mild reaction conditions, diverse protecting group options, the ability to purify intermediates, and ease of scaling up production. It remains widely used for synthesizing peptides containing fewer than 10 amino acids.

Looking ahead, with continued progress in synthetic chemistry and biotechnology, liquid-phase synthesis is expected to undergo further optimization toward automation, high-throughput operations, and greener chemistry—potentially leading to more efficient and environmentally friendly peptide synthesis processes in the future.

2. Solid-Phase Peptide Synthesis (SPPS)

Solid-phase peptide synthesis (SPPS), a revolutionary methodology introduced in 1963 by Nobel laureate Robert Bruce Merrifield, effectively overcame the purification limitations inherent in traditional liquid-phase synthesis. In 1965, Merrifield further advanced the field by designing the first automated peptide synthesizer. The core innovation of SPPS involves covalently anchoring the C-terminus of the growing peptide chain to an insoluble solid support, such as

polystyrene resin. Subsequent amino acid coupling reactions are then carried out sequentially on the solid support. After each reaction step, unreacted reagents and by-products can be efficiently removed through simple washing and filtration, eliminating the need for complex liquid-phase separation and greatly streamlining the peptide synthesis process.

In a landmark 1969 paper published in the *Journal of the American Chemical Society*, Merrifield's team reported the successful total chemical synthesis of ribonuclease A—a protein comprising 124 amino acids—using SPPS. This achievement powerfully demonstrated the feasibility and potential of the technique. In 1984, Merrifield was awarded the Nobel Prize in Chemistry "for his development of methodology for chemical synthesis on a solid matrix." The Nobel Committee emphasized in its announcement: "*This methodology has created new possibilities in the field of peptide and protein research and has already yielded results of great practical value*." SPPS has since become a paradigm-shifting technology at the intersection of chemistry and biology and is widely regarded as the cornerstone method for peptide synthesis. In its 2004 obituary of Merrifield, *Nature*unequivocally stated: "*The solid-phase approach is now the standard method for synthesizing peptides.*"

Since its inception, SPPS has undergone systematic innovation and refinement. In terms of protecting group strategies, a major advance came in 1972 when Lou Carpino introduced the 9-fluorenylmethoxycarbonyl (Fmoc) group for α-amino protection. The Fmoc group can be rapidly removed under mild basic conditions (e.g., 20% piperidine in DMF, typically within 10 minutes), avoiding the need for strong acidic treatments (such as anhydrous TFA) required in the t-Boc strategy. This greatly enhanced compatibility with acid-sensitive groups (e.g., the indole ring of tryptophan or glycosylation modifications) and significantly expanded the applicability of SPPS.

Built on the orthogonal Fmoc/t-Boc protection strategy, various automated peptide synthesizers have been developed and continuously upgraded. Concurrently,

solid-phase resins have been optimized, new peptide coupling reagents have been devised, and amino acid side-chain protecting groups have been designed with increasing sophistication. These breakthroughs have collectively transformed SPPS from a manual laboratory technique into a highly automated, high-throughput standard synthesis method, now widely used in numerous cutting-edge fields such as biomedicine, materials science, and proteomics.

3. Principle and Key Steps of Solid-Phase Peptide Synthesis

3.1 Selection and Functional Design of Solid Supports

The solid support serves as a critical foundation in SPPS, directly influencing synthesis efficiency and product quality. An ideal solid support should possess the following characteristics: high chemical stability to withstand various acids, bases, and organic reagents used in SPPS; good swelling properties to ensure sufficient diffusion of reagents within the support for efficient reactions; and modifiable functional groups to form stable covalent linkages with amino acids.

Currently, two main types of supports are widely used:

• Polystyrene-divinylbenzene (PS-DVB) resin: One of the earliest and most commonly used solid supports in peptide synthesis. Its high cross-linking degree provides excellent mechanical strength and chemical inertness. However, due to its relatively poor swelling capacity and high rigidity, it is more suitable for synthesizing short to medium-length peptides.

• Polyethylene glycol-based resins (e.g., PEG-PS resin): These resins are often functionalized with linkers such as Rink amide or Wang carboxylic acid. The PEG chains significantly enhance hydrophilicity by reducing the contact angle, effectively disrupting π-π stacking of hydrophobic peptide chains and suppressing β-sheet aggregation. Combined with a low-loading design (0.3–0.6 mmol/g), this type of support can increase the yield of long peptides (>50 amino acids) to over 40%, making it the preferred choice for synthesizing long and complex modified peptides.

3.2 Amino Acid Coupling and Protecting Group Strategies

At the outset of solid-phase peptide synthesis (SPPS), the first amino acid must be attached to the solid support. The most widely used strategy today is the Fmoc/tBu protection scheme. In this approach, the first amino acid, with its α-amino group protected by Fmoc, is covalently linked via its carboxyl group to a functional group on the resin—typically forming an ester bond. Likewise, the α-amino groups of all subsequently added amino acids are also Fmoc-protected to prevent undesired side reactions during the coupling steps.

Throughout the synthesis, reactive side-chain functional groups (e.g., hydroxyl, thiol, amino, and carboxyl groups) must also be protected using stable protecting groups such as tBu, Boc, Trt, or Pbf. These side-chain protecting groups must fulfill two essential requirements: they must remain stable under the mild basic conditions (e.g., piperidine/DMF) used for repeated Fmoc deprotection, yet be efficiently and completely removed during the final cleavage step, which typically employs strong acidic conditions.

A cornerstone of SPPS is the orthogonal protecting group strategy. This involves selecting protecting groups that can be cleaved under specific and mutually compatible conditions, allowing independent removal of the α-amino protecting group, side-chain protections, and the anchor linking the peptide to the resin. This precise control ensures the accurate stepwise assembly of the peptide chain.

3.3 Deprotection and Amino Acid Coupling Cycle

The stepwise elongation of the target peptide chain is achieved through repeated cycles of deprotection, washing, coupling, and subsequent washing.

• Deprotection: Each cycle begins by treating the resin with a solution of 20–50% piperidine in DMF to selectively remove the Fmoc protecting group from the α-amino terminus of the growing peptide chain. This reaction proceeds rapidly, exposing a free α-amino group. The resin must then be thoroughly washed to remove the deprotection by-products and any residual piperidine.

• Amino Acid Coupling: The next Fmoc-protected amino acid (with appropriate side-chain protection) is introduced to the reaction. To drive efficient amide bond formation between its carboxyl group and the newly exposed α-amino group, the carboxyl group must be activated. This is typically achieved using coupling reagent systems—such as DIC/HOAt, HCTU/HOAt/DIEA, or HBTU/HOAt/DIEA dissolved in solvents like DMF.

• Post-Coupling Treatment: After the coupling reaction is complete, the resin is again washed extensively (commonly with DMF and other solvents) to remove excess reagents and by-products, preparing it for the next cycle.

3.4 Cleavage and Purification of the Peptide

Once the full-length peptide chain has been assembled on the solid support, it must be cleaved from the resin and simultaneously deprotected to yield the free, biologically active peptide. This is generally accomplished using a cleavage cocktail containing a high concentration of trifluoroacetic acid (TFA), along with various scavengers such as water, thioanisole, triisopropylsilane (TIS), and 1,2-ethanedithiol. The cocktail cleaves the anchor linking the peptide to the resin and also removes all side-chain protecting groups (e.g., tBu, Boc, Trt, Pbf).

The cleavage reaction is typically carried out at room temperature for 1–4 hours. The resin is then removed by filtration, and the peptide-containing solution is precipitated by adding cold diethyl ether. The resulting crude peptide precipitate is collected via centrifugation or filtration. Finally, the crude product is purified—often by high-performance liquid chromatography (HPLC)—using gradient elution with buffers containing TFA or formic acid, yielding the target peptide in high purity.

4. Optimization of Solid-Phase Peptide Synthesis Technology

4.1 Refined Design of Protecting Group Strategies

The choice of protecting group strategy directly influences the selectivity, efficiency, and final purity and integrity of the synthesized product, making it one of

the core elements of peptide synthesis. The key objective is to achieve strict orthogonality—ensuring that specific protecting groups can be selectively and efficiently removed at the desired stage without affecting other protecting groups or the backbone structure. Current optimization efforts focus on: developing novel protecting groups that can be cleaved under milder and more specific conditions to minimize potential chemical damage to peptides containing unstable modifications or complex structures; and ensuring that sensitive groups in complex peptides—such as those containing non-natural amino acids or post-translational modifications—remain stable throughout the synthesis while still being precisely removable.

4.2 Optimization of Efficient Condensation and Coupling Strategies

The efficiency and quality of amino acid coupling are critical for successful peptide chain elongation. Research is also focused on developing coupling reagent systems that exhibit high reactivity, low racemization risk, and minimal by-product formation. For long, hydrophobic, or difficult sequences, further refinement of coupling strategies is essential to improve synthesis efficiency and product quality.

4.3 Performance Optimization and Innovation of Solid Supports

The properties of the solid support directly affect reaction kinetics and peptide conformation. Key optimization pathways include: enhancing hydrophilicity, swelling capacity, and site accessibility through functionalization to suppress peptide aggregation; and developing systems with rapid separation characteristics or mild cleavage mechanisms to avoid structural damage during peptide release, along with the design of novel solid supports.

5. Advantages and Limitations of SPPS Technology

The core advantage of SPPS lies in its use of a solid support to enable efficient synthesis and simplified purification, compatibility with automated synthesis (e.g., fully automated peptide synthesizers), and the achievement of high yield (>80%) and high purity in the synthesis of short peptides (<30 amino acids). However, several

limitations remain: when the peptide chain exceeds 50 amino acids, steric hindrance and accumulated side reactions lead to a sharp decline in coupling efficiency and an increase in deletion peptides; hydrophobic peptide sequences tend to form diffusion barriers due to β-sheet stacking, significantly hindering subsequent couplings—existing modification strategies only partially alleviate this issue; the high unit cost of protected amino acids, efficient coupling reagents, and functionalized resins restricts large-scale applications, while the disposal of organic waste further increases economic and environmental pressures.

6. Applications and Expansion of SPPS

6.1 Broad Applications in Biomedicine

SPPS serves as the core technology for the industrial production of clinical peptide therapeutics. Numerous peptide drugs synthesized via SPPS have been successfully marketed, such as somatostatin (14-mer), octreotide (8-mer analog), and leuprolide (9-mer), with indications covering oncology and endocrine disorders. The high-throughput synthesis capability of SPPS enables rapid construction of peptide analog libraries, accelerating lead compound screening. In vaccine development, SPPS is used to synthesize conformationally constrained antigenic epitope peptides. These can be lipidated or polymerized to enhance immunogenicity for epitope-based vaccines. In diagnostics, epitope-mimetic peptides are employed to develop highly sensitive ELISA and SPR biosensors.

6.2 An Essential Tool in Protein Science

SPPS allows precise synthesis of specific peptide sequences to mimic functional domains of proteins. By analyzing their interactions with ligands/receptors, it helps elucidate protein structure-function relationships. Key applications include:

• Post-translational modification (PTM) studies: synthesizing peptides containing modification sites such as pTyr/pSer/pThr (phosphorylation) and AcK (acetylation) to decipher how modifications regulate conformation and activity;

• Binding site identification: constructing random/truncated peptide libraries to screen high-affinity ligands and map protein active pockets and allosteric sites.

Additionally, SPPS facilitates chemical protein synthesis and modification. Combined with native chemical ligation (NCL) or expressed protein ligation (EPL), it enables total chemical synthesis of proteins under 200 amino acids, allowing precise incorporation of non-canonical groups—including unnatural amino acids, $^{13}C/^{15}N$ isotopic labels (for NMR analysis), and photo-crosslinkers (e.g., diazirine). C-terminal or site-specific side-chain modifications can also be introduced directly during synthesis, such as FITC (for fluorescence) or biotin (for affinity purification), supporting protein tracking and interaction studies.

6.3 Innovative Applications in Materials Chemistry

6.3.1 Self-Assembling Peptide Materials

Designed amphiphilic peptides (e.g., EAK16-II, RADA16) can undergo hierarchical self-assembly under physiological ion strength or pH via intermolecular β-sheet hydrogen bonding and electrostatic complementarity, forming nanofiber networks that crosslink into porous hydrogels (pore size: 50–200 nm; storage modulus $G' > 1$ kPa). Using SPPS to precisely control the ratio of hydrophobic/hydrophilic residues and charge distribution allows programmable tuning of gel mechanical properties and degradation rates. When used as biomimetic extracellular matrix scaffolds in tissue engineering, these materials require embedded RGD cell-adhesion motifs (e.g., GGG-RGDSP) to mediate cell adhesion, often in combination with loaded growth factors (e.g., TGF-β) to effectively promote tissue regeneration.

6.3.2 Antimicrobial Peptide Coatings and Surface Modification

Cationic α-helical antimicrobial peptides (e.g., LL-37, magainin-2) synthesized via SPPS can be covalently immobilized on medical device surfaces (e.g., titanium joints, silicone catheters) using thiol-ene click chemistry or dopamine-inspired deposition. Their antimicrobial mechanism relies on the

amphipathic structure inserting into bacterial lipid bilayers and forming transmembrane pores leading to cytoplasmic leakage—rather than inhibiting bacterial adhesion. Although such coatings exhibit selective membrane-lytic activity, they face challenges in vivo such as inactivation by plasma protein adsorption. Solutions include surface modification with phosphorylcholine or PEG brushes to reduce biofouling and maintain antimicrobial activity for over 72 hours.

6.4 Convergence of SPPS with Cutting-Edge Technologies

Enzymatic Solid-Phase Synthesis: Integrating enzymatic ligation into solid-phase strategies using enzymes such as sortase A or engineered protease variants (e.g., subtiligase) enables mild and efficient peptide fragment coupling on solid supports (e.g., via immobilized glycine spacers). This approach reduces the use of highly corrosive coupling reagents (e.g., carbodiimides) by 80–90% and suppresses racemization to <1% at native L-amino acid ligation sites. It is particularly advantageous for synthesizing complex modified peptides (e.g., cyclics, lipopeptides) and protein semisynthesis—e.g., using sortase A to catalyze the directional coupling of solution-phase bioactive fragments to solid-phase targeting peptides, enabling precise construction of chimeric molecules and offering a greener, high-fidelity route for challenging syntheses.

Microfluidic Chip Integration with SPPS: Microfluidic technology offers miniaturization, high throughput, and precise reaction control. Its integration with SPPS enables miniaturized and high-throughput peptide synthesis. Within microfluidic chips, controlled fluid flow and reaction timing in microchambers allow efficient solid-phase synthesis. This integrated system not significantly reduces reagent consumption and waste generation but also enables real-time monitoring and control, providing an efficient platform for constructing large, diverse peptide libraries.

Computer-Aided Design and SPPS Automation: Computer-aided design (CAD) tools allow researchers to rapidly design optimal synthetic routes based on

structural and functional requirements of target peptides. CAD can predict potential synthesis challenges—especially for “difficult sequences”—such as coupling failures, low efficiency, or aggregation, and suggest optimized strategies (e.g., adjusting protection schemes, selecting special activators/solvents, or extending reaction times). Mature CAD technologies are now deeply integrated with automated peptide synthesizers, enabling full automation from sequence design to synthesis and further improving the efficiency and reliability of SPPS—paving the way toward intelligent and precision peptide synthesis.

7. Conclusion and Outlook

Solid-phase peptide synthesis (SPPS), as a foundational technology in peptide chemistry, has undergone more than half a century of development and refinement. It now serves as a critical bridge connecting chemistry, biology, materials science, and other disciplines. While SPPS still faces multiple challenges in the synthesis efficiency of long peptides, difficulties in hydrophobic peptide synthesis, and cost control, ongoing innovations in protecting group strategies, the development of novel coupling reagents and solid supports, as well as deep integration and convergence with cutting-edge technologies such as AI-driven optimization, are steadily advancing SPPS toward a more efficient, precise, and environmentally sustainable future.