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Streamlined Solid-Phase Synthesis of Peptide–Oligonucleotide Conjugates via Direct Elongation

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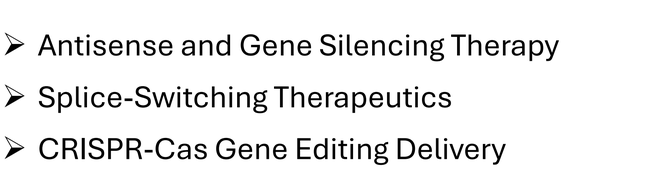
Where molecules come together

Introduction

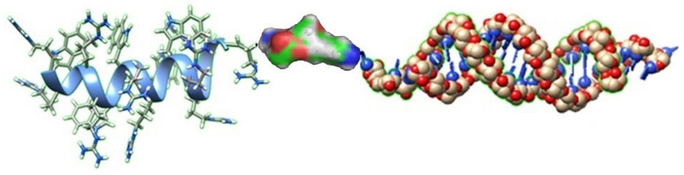
Peptide–oligonucleotide conjugates (POCs) are promising platforms for therapeutic and diagnostic applications, but conventional synthesis is often limited by multistep fragment coupling and difficult purification. Here, we report an automated solid-phase approach for sequential assembly of POCs on a single support. An Fmoc-protected peptide was synthesized on controlled-pore glass, followed by direct oligonucleotide elongation using standard phosphoramidite chemistry without an added linker. Final cleavage from the resin and global deprotection were performed using a custom cocktail. RP-HPLC and LC-MS analyses confirmed efficient coupling at each stage, with stepwise yields exceeding 95% and retention of sensitive residues and oligonucleotide integrity. This one-resin strategy simplifies POC synthesis, improves efficiency, and offers a scalable alternative to conventional fragment-based methods.

Different types of Peptide–Oligonucleotide Conjugates

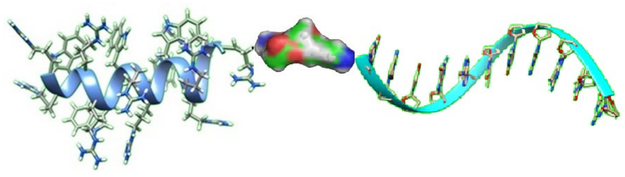
CPP-mono-dsDNA



CPP-trilinker-dsDNA



CPP-trilinker-ssDNA



Importance of peptide oligo conjugate

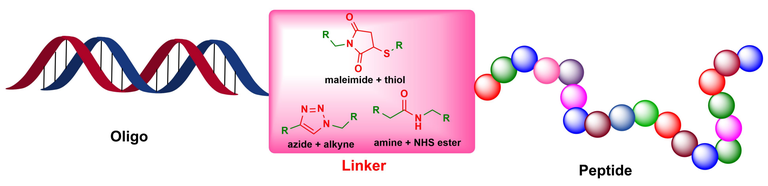
- Antisense and Gene Silencing Therapy
- Splice-Switching Therapeutics
- CRISPR-Cas Gene Editing Delivery
- Targeted Oncology and Tumor Imaging
- Antibody-Oligonucleotide Conjugates (AOCs)
- Biosensing and Molecular Diagnostics

Advantages of in line oligo peptide synthesis over conventional post conjugation method

- One Build. One Purification.
- Faster, Cleaner Coupling
- Precision 1:1 Conjugates
- Linker Design Freedom
- No Aggregation. No Solubility Mismatch.
- Can be used for the synthesis of cyclic peptides
- Fluorophore also can be as desired

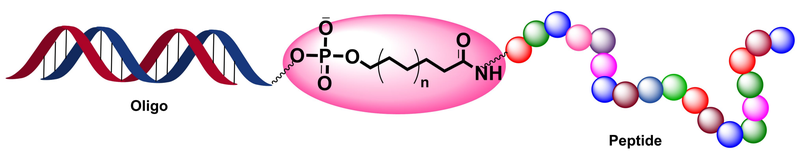
Different types of peptide oligo conjugations

1. Conventional peptide-oligo synthesis using post conjugation



Separately synthesize, deprotect, and purify both fragments, then conjugate them in solution using mutually orthogonal chemistry.

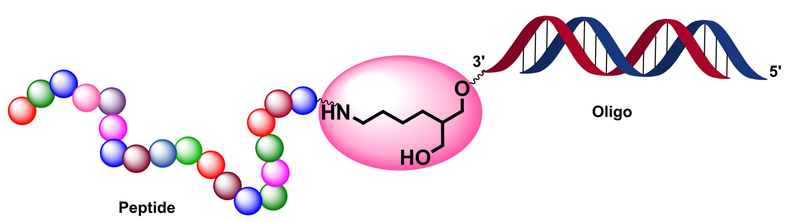
2. Direct elongation: N terminal of peptide on 3’ or 5’ of Oligo using monolinker



5’ Oligo 3’ Linker - Peptide Conjugation; linear

Using monofunctional linkers, the peptide chain can be assembled efficiently through in-line solid-phase synthesis. A hydroxy linker can then be used to continue the oligo synthesis, after which the CPG support and protecting groups are removed to yield the final product.

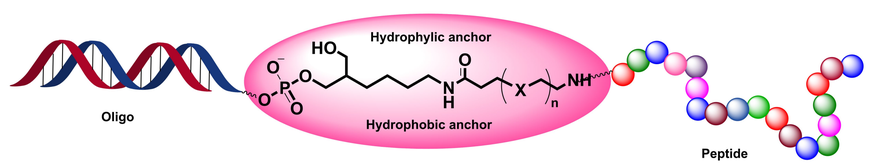
3. Direct Elongation: C terminal of peptide on 3’ Oligo using trilinker



5’ Oligo 3’ – Linker – (C – N terminal) Peptide conjugation

Using trifunctional linkers, in-line solid-phase synthesis can be performed efficiently. After synthesis, the CPG support and protecting groups can be removed, allowing the final product to be obtained with a single purification step.

In line POC synthesis using Biosyn linkers



Direct elongation: C terminal of peptide on 3’ Oligo using trilinker with different anchors

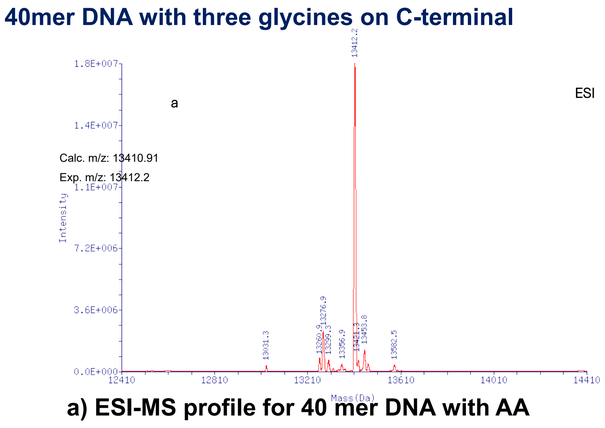
Beyond the ease of synthesis and purification associated with using a single construct, modified linkers—whether hydrophilic or hydrophobic—allow the peptide chain to be fine-tuned for specific downstream applications.

References

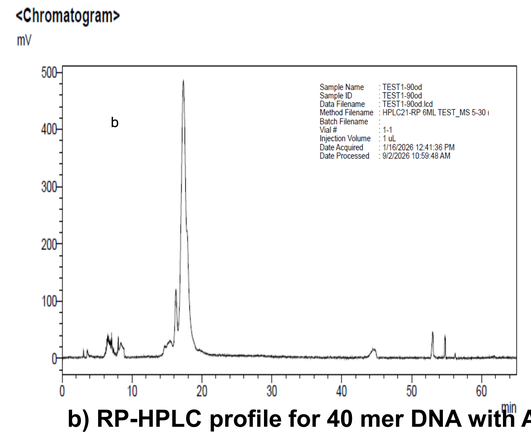
1) Stetsenko et al. Molecules 2021, 26, 5420; 2) Bose et al., Curr. Issues Mol. Biol. 2024, 30, 11031-11047; 3) Smietana et al. Chem. Eur. J., 2024, 30, e202401069; 4) Gothelf et. al. Nucleic acid Research, 2024, 52, 49-58; 5) Tian et al. Chem. Comm., 2023, 59, 5839-5842; 6) Obika et al. J. Org. Chem., 2026, 91, 7758-7768; 7) Adler et al. Curr. protoc, 2025, 5, e70154.

Analytical Characterization of Oligonucleotide–Peptide Conjugates Prepared by Direct Solid-Phase Elongation

40mer DNA with three glycines on C-terminal

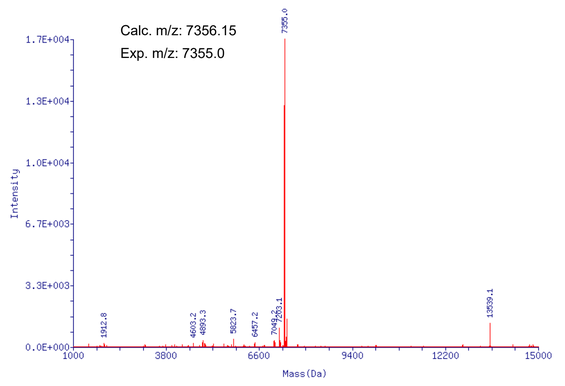


a) ESI-MS profile for 40 mer DNA with AA

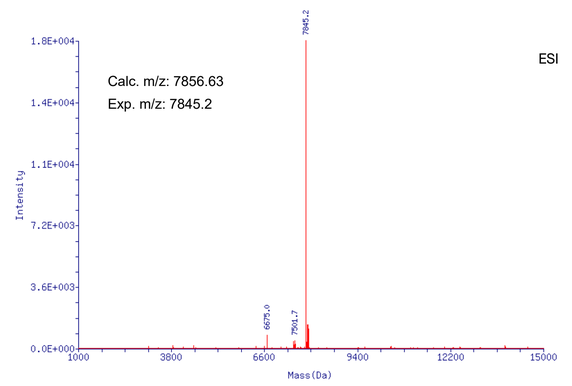


b) RP-HPLC profile for 40 mer DNA with AA

Crude ESI profile for the synthesis of 20 mer DNA and 20mer RNA (2’ OMe and 2’ F) with six different amino acids



Crude ESI profile for 20 mer DNA with AA



Crude ESI profile for 20 mer RNA (2’ OMe and 2’ F) with AA

Conclusions

On-CPG peptide–oligonucleotide synthesis offers a simple, flexible way to prepare well-defined peptide–oligo conjugates directly on a single solid support. By combining peptide assembly and oligonucleotide processing in one workflow, it reduces intermediate handling, purification steps, and material loss compared with solution-phase conjugation.

Its success depends on selecting compatible protecting groups, linkers, and mild deprotection conditions to maintain both peptide and oligonucleotide integrity. Overall, this platform can accelerate preparation of targeted and delivery-focused conjugates, with further optimization supporting more complex designs and future scale-up.

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