EPS 2026 | O08

Green, DMF-Free ASPPS

Beyond Dissolution

Nanoassembly-Enabled Aqueous Solid-Phase Peptide Synthesis

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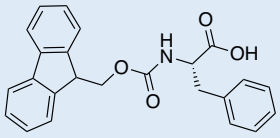
The idea

***Beyond Dissolution**: the substrate builds the reaction field*

We discovered that in-water mixing of Fmoc-amino acids, bases, and coupling reagents produces nanoassemblies.

Amphiphilic Fmoc-amino acids

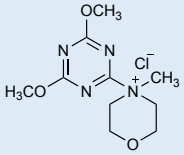
Hydrophobic Fmoc group & hydrophilic COOH
Surfactant-like structure



Organic Base



Water soluble coupling reagents



① Mix two components in water to obtain a clear solution
② Add the water-soluble coupling reagent

Three-component

Nanoassembly

Self-assembled dynamic reaction field

The standard Fmoc-AA-OH toolbox can be used in water

We thought that a reaction field might have been formed inside.
If this is the case, the reaction should be accelerated in water.

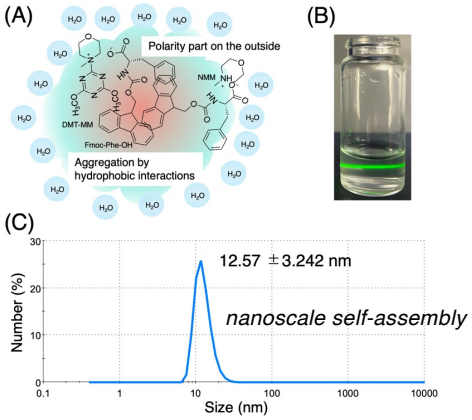


Fig. 1 Aqueous three-component nanoassemblies of Fmoc-Phe-OH with DMT-MM and NMM.
(A) Schematic diagram of nanoassemblies;
(B) (B) Tyndall effects by nanoassemblies;
(C) Particle size distribution for nanoassemblies

PDI is consistently high across amino acids — indicating that a range of assembly sizes coexist, rather than a single defined structure. The measured size itself is not the determinant of reactivity; DLS instead confirms that hydrophobic self-assembly is occurring.

RSC Adv. 2026, **16**, 18073. Patents: JP2022-208765; WO2024-143052.

Proof of concept

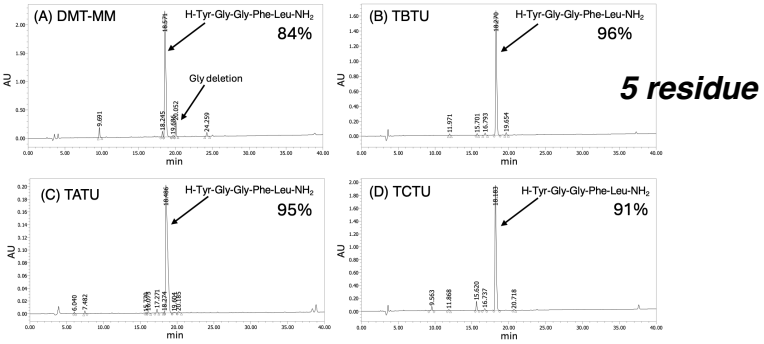
Nanoassembly-enabled ASPPS

We next applied this chemistry to peptide synthesis.

Table 3. The protocols for ASPPS using nanoassemblies of Fmoc amino acids.

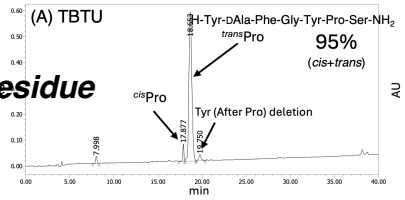
SP Wave
Biotage® Initiator+

Step	Reagents	Volume (ml)	Temp (°C)	Time
Wash	Water	5	25	3 min × 5
Coupling reaction	Nanoassemblies of Fmoc-amino acids with coupling agent and base	6	75	10 min
Wash	Water	5	25	3 min × 5
Wash	EtOAc	5	25	3 min × 3
Deprotection	20% pip-EtOAc	5	25	20 min
Wash	EtOAc	5	25	3 min × 3



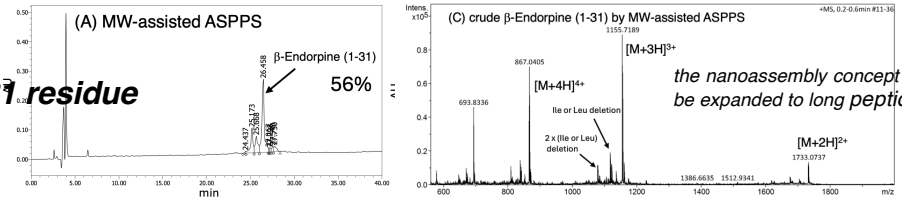
5 residue

Fig. 2 HPLC profiles of crude Leu-enkephalin amide by ASPPS using nanoassemblies Fmoc-amino acids with (A) DMT-MM and NMM; (B) TBTU and DIEA; (C) TATU and DIEA; (D) TCTU



7 residue

Fig. 3 HPLC profiles of crude dermorphin by ASPPS using nanoassemblies Fmoc-amino acids with TBTU and DIEA.



31 residue

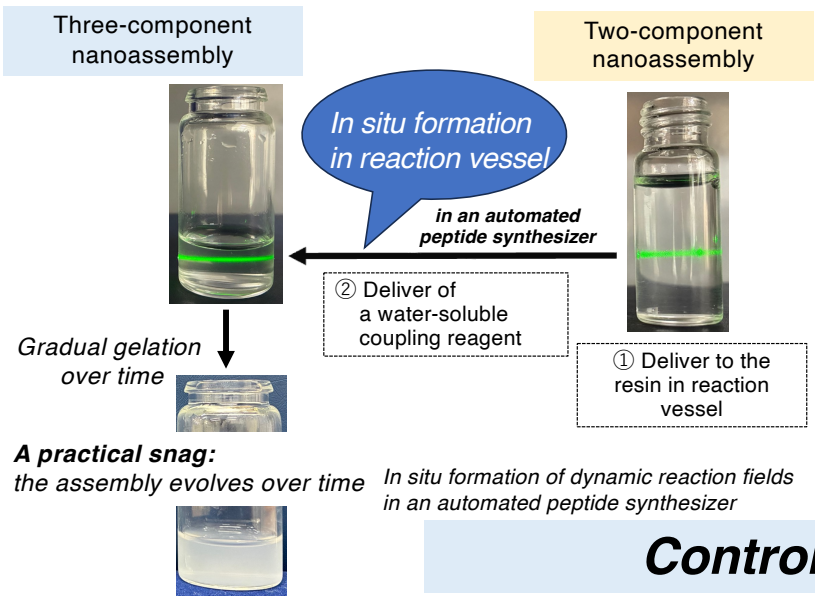
the nanoassembly concept could be expanded to long peptides.

Fig. 4 HPLC profiles of crude b-Endorphin (1-31) obtained by ASPPS employing nanoassemblies. (C) ESI-MS (TOF) spectra of crude b-Endorphin (1-31) obtained by ASPPS.

The next problem and solution

Controlled nanoassembly for automation

Although the chemistry worked well, we encountered a practical problem for automation.



In situ formation of dynamic reaction fields in an automated peptide synthesizer

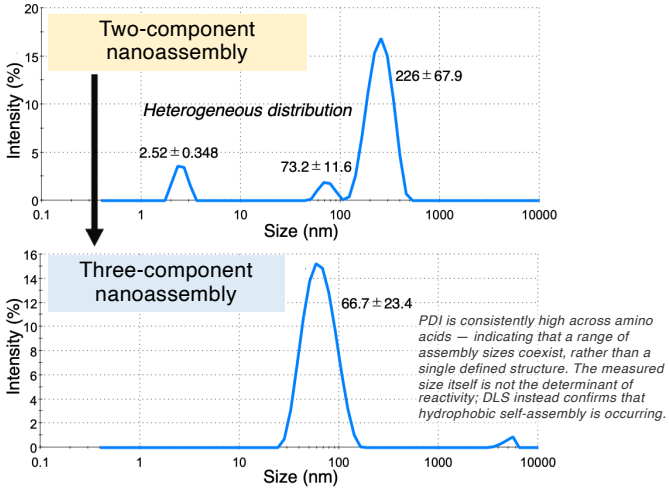


Fig. 5 DLS of two- and three-component nanoassemblies.

Controlled nanoassembly

The reaction field is not stored — it is generated on demand through controlled nanoassembly.

RSC Adv. 2026, **16**, *in press.* Patents: WO2024-143052; JP2025-143052

Demonstration

Fully automated ASPPS enabled by nanoassemblies

We then evaluated this ASPPS automated program.

Even at this level, aqueous peptide synthesis remained feasible.

5 residues

Leu-enkephalin amide

11 residues

Substance P

37 residues

GLP-1 (1-37)

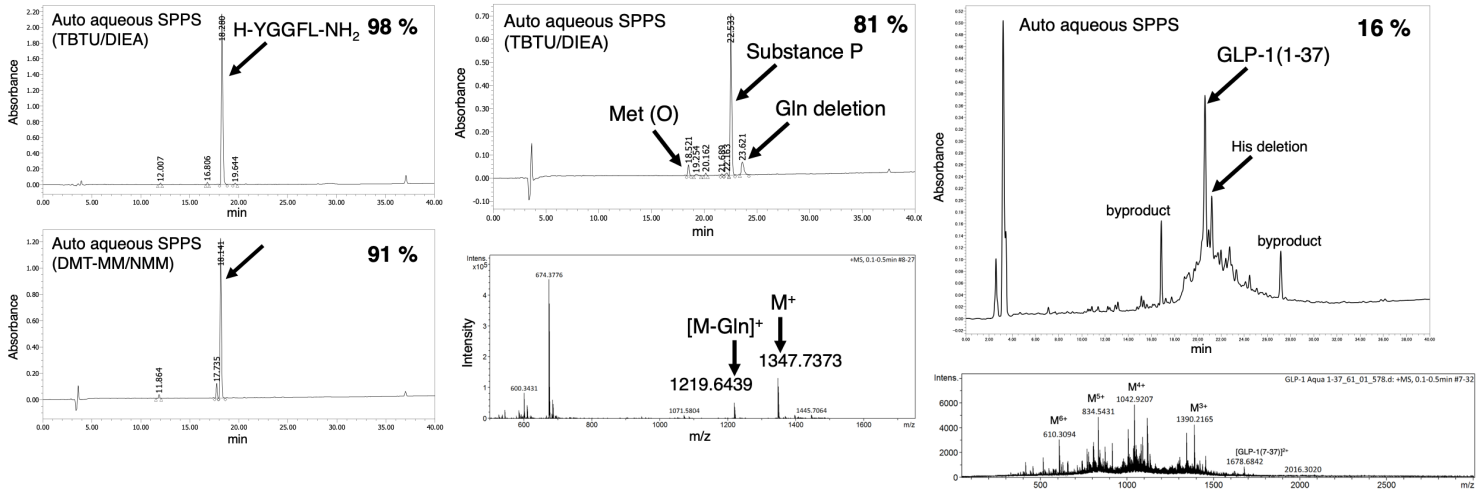


Fig. 6. Product analysis of Leu-enkephalin amide, substance P, and GLP-1 (1-37) synthesized by fully automated ASPPS.

Overall, controlled nanoassemblies really do work for fully automated aqueous SPPS.

CONCLUSION

Beyond Dissolution: water, the architect of the reaction field

We set out to replace DMF with water. What we found was something more fundamental — Water is not simply a replacement solvent.

Through self-assembly of substrates, water builds a reaction field. It concentrates the substrates within a hydrophobic microenvironment, creating conditions that favor coupling.

DMF-free | Fully automated | Beyond 30 residues | Green by design