

# Co-assembly driven high-yielding enzymatic peptide synthesis in water

Ameena Yoonus,<sup>1,2</sup> Kübra Kaygisiz,<sup>2</sup> Rinat Abzalimov,<sup>2</sup> Anna Geissmann,<sup>1,2</sup> Ankit Jain,<sup>1,3</sup>  
Rein V. Ulijn<sup>1,2</sup>

1. Ph.D. Program in Chemistry, The Graduate Center of the City University of New York, New York, NY 10016, USA

2. Advanced Science Research Center (ASRC), City University of New York, 85 St. Nicholas Terrace, New York, NY 10031, USA

3. Department of Chemistry, Brooklyn College of the City University of New York, Brooklyn, New York 11210, USA

---

Biological systems achieve complex, regulated functions by coupling molecular recognition and self-assembly.<sup>1</sup> The peptide bond is central to this, yet forming it in water has been challenging because hydrolysis is thermodynamically favoured. Previous approaches relied on activated substrates or on the assembly of the product to shift this equilibrium.<sup>2,3</sup> Here, we ask whether co-assembly of unactivated substrates can create a local environment that drives peptide bond formation in water.

We report a dynamic peptide system in which sequence-specific interactions govern the reaction network evolution and supramolecular pathway selection. We create reaction networks using the Dynamic Peptide Library (DPL) approach, where the enzyme thermolysin catalyzes the reversible breaking and formation of amide bonds, generating a mixture of peptides, from which specific sequences are selectively amplified.<sup>4</sup> Our design consists of a modified dipeptide sequence with a fatty acid chain (C<sub>8</sub>) on the N-terminus that undergoes hydrolysis due to the endoprotease activity of thermolysin and interacts supramolecularly to co-assemble with different tryptophan-containing dipeptides, undergoing different supramolecular pathways. We have a supramolecular system that can control the pathway via a single peptide mutation in the dipeptides. We observe that, remarkably, in the case of Arginine-containing dipeptides, it undergoes Liquid-Liquid Phase Separation to form droplets that act as microreactors for peptide synthesis, leading to higher-order sea urchin-like structures in 79% yield. Whereas in a Lysine-containing dipeptide, it bypasses compartmentalization, leading to direct aggregation; in the Aspartic acid system, it suppresses assembly. Furthermore, we have shown that the position of Arginine also matters in which pathway it chooses. The dipeptide therefore acts as both substrate and supramolecular regulator. This minimal system shows sequence-regulated reaction-assembly pathways and points towards sustainable, high-yielding peptide synthesis in water without coupling reagents.

## References:

(1) Vantomme, G.; Meijer, E. W. *Science* **2019**, *363* (6434), 1396–1397. (2) Williams, R. J.; Smith, A. M.; Collins, R.; Hodson, N.; Das, A. K.; Ulijn, R. V. *Nat. Nanotechnol.* **2009**, *4* (1), 19–24. (3) Toledano, S.; Williams, R. J.; Jayawarna, V.; Ulijn, R. V. *J. Am. Chem. Soc.* **2006**, *128* (4), 1070–1071. (4) Pappas, C. G.; Shafi, R.; Sasselli, I. R.; Siccardi, H.; Wang, T.; Narang, V.; Abzalimov, R.; Wijerathne, N.; Ulijn, R. V. *Nat. Nanotechnol.* **2016**, *11* (11), 960–967.