Understanding aggregation during peptide synthesis using in-line UV analysis

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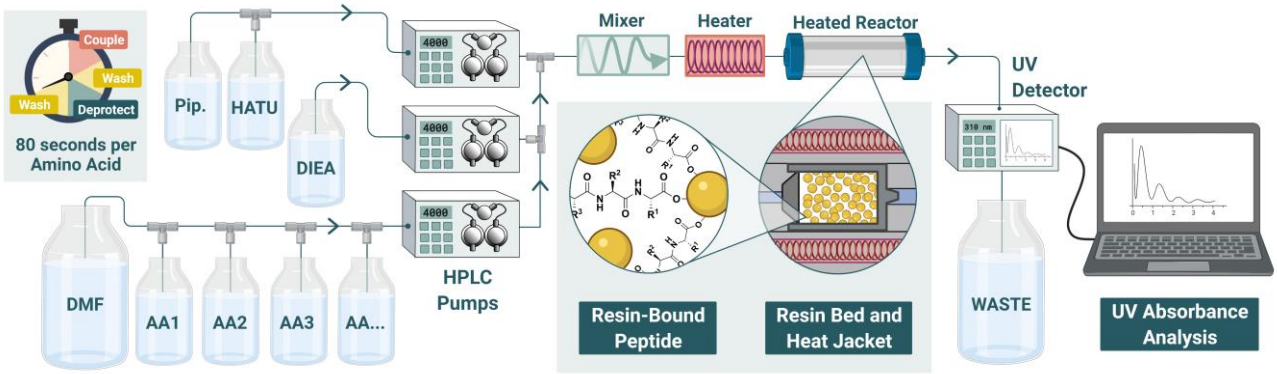
In 2021, 80 peptide drugs were available on the global market, and this number is expected to increase significantly in the incoming years.[1] With more than 150 peptides in clinical development and 500 peptides undergoing preclinical studies, the need for more efficient, reliable, and optimized peptide synthesizers is even more pressing. The use of the first flow-based peptide synthesizers dates back to the early 80s.[2,3] In the recent years, advanced fast-flow peptide synthesizers were developed, allowing for automated and efficient couplings that can be optimized by time-resolved monitoring.[4-6] However, sequence-dependent aggregation is still one of the main reasons for unsuccessful peptide synthesis.



The introduction of time-resolved reaction monitoring, relying on the Fmoc deprotection trace, gives access to valuable data such as the aggregation during synthesis and the efficiency of each coupling. Relying on this in-line data and after postulating that aggregation is dependant on the sequence with an increased effect of the first few amino acids, we investigated the impact of the linker in-between the resin and the peptide itself and tried to determine its role in aggregation formation.

The end goal of this study is to give peptide chemists a better understanding of the causes that lead to aggregation. This phenomenon is still one of the biggest challenges for peptide synthesis and tackling it would allow not only for the obtention of better yields and purity, but also for the possibility to understand the origin of the so-called “difficult couplings”.

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