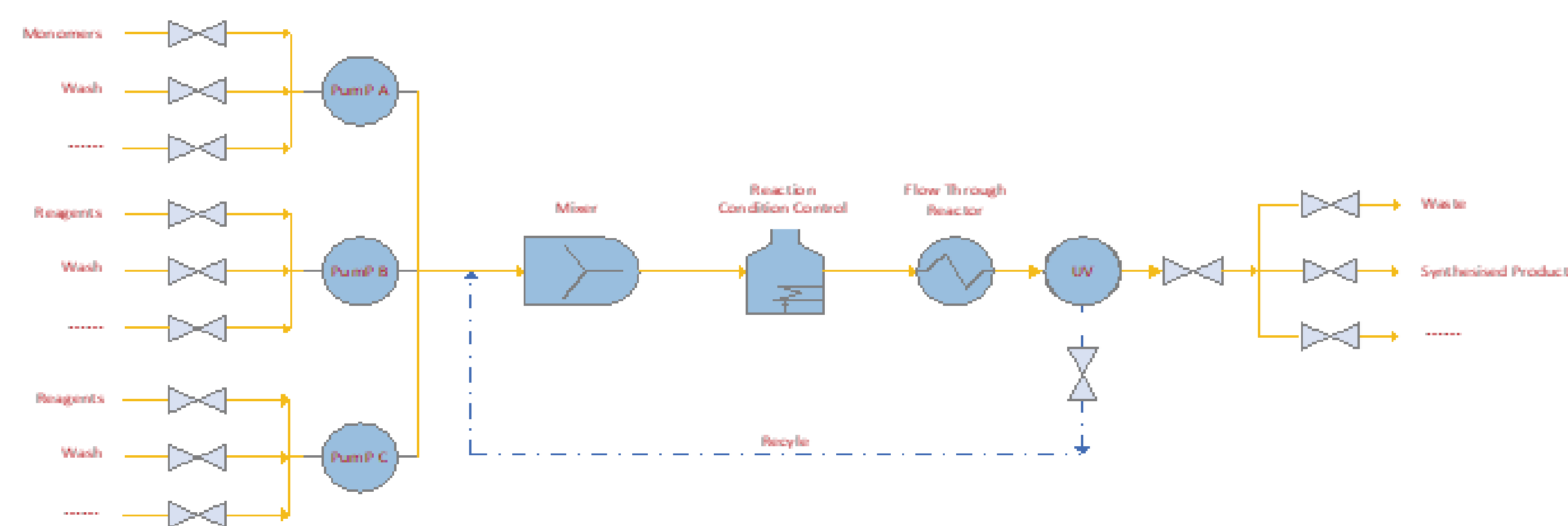
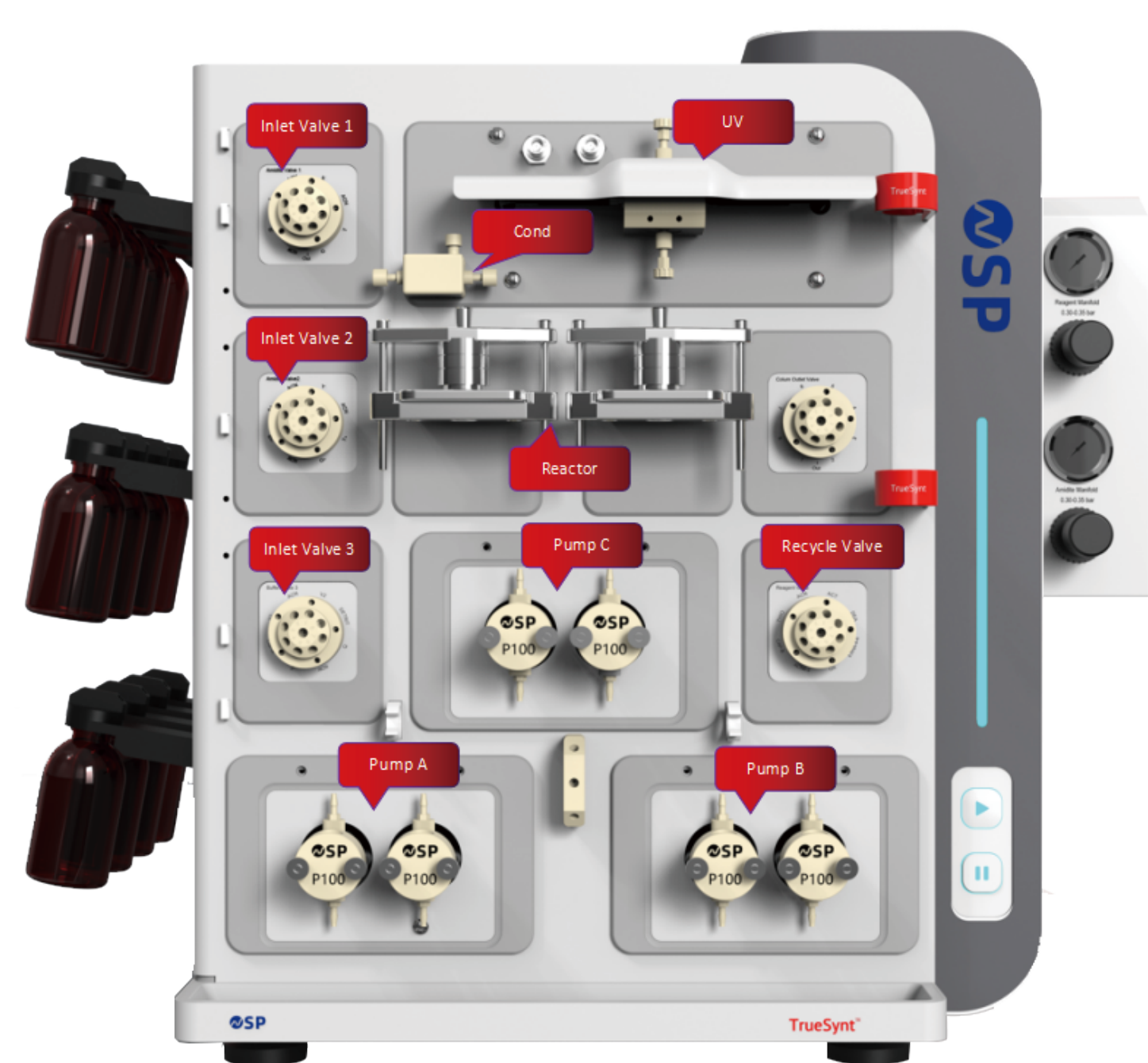


Abstract: TrueSynt is an advanced multifunctional peptide synthesizer capable of rapidly producing complex linear peptides, cyclic peptides, and modified peptides. It employs solid-phase high-temperature flow synthesis technology, combined with a material recycling module, to control the assembly time of amino acid monomers to around 3 minutes and the equivalent control to below 5 equivalents, thereby reducing synthesis costs. Additionally, with proprietary linker and cyclization technology, it can achieve single-shot rapid synthesis of natural cyclic peptide molecules, reducing synthesis time from several days to minutes, with crude product purity reaching over 95%. Furthermore, this technology is compatible with 20 types of natural amino acids as well as non-natural amino acids, enabling efficient and high-purity production ranging from 6-membered rings to 39-membered rings.

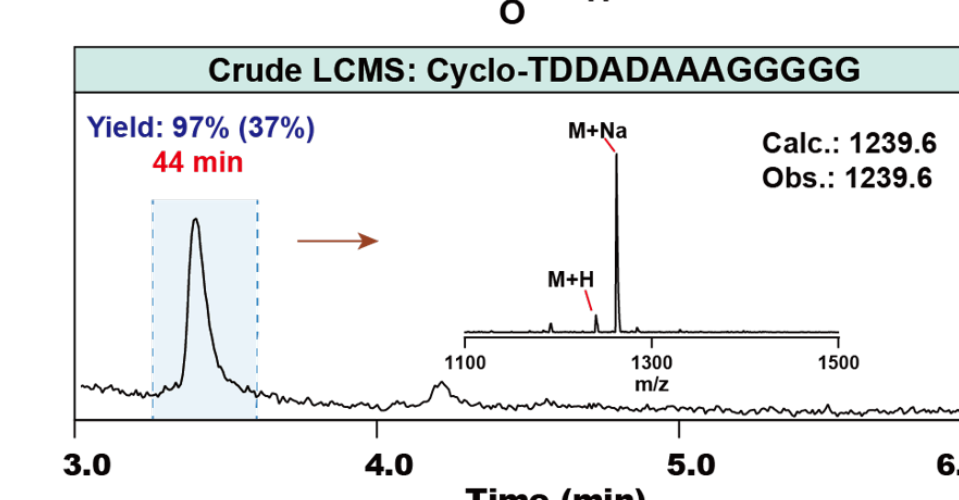
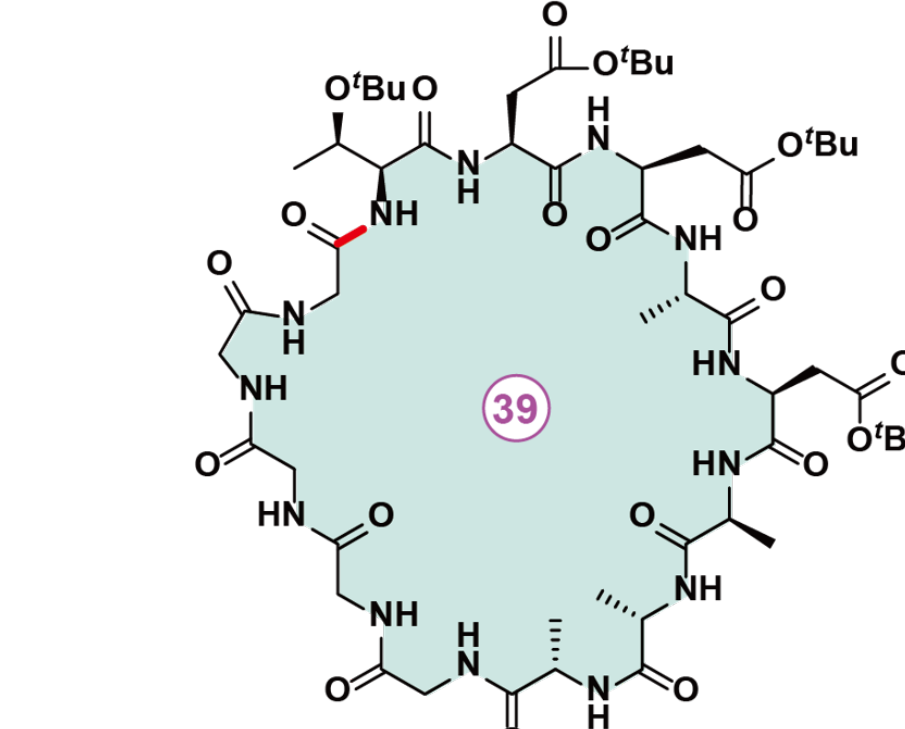
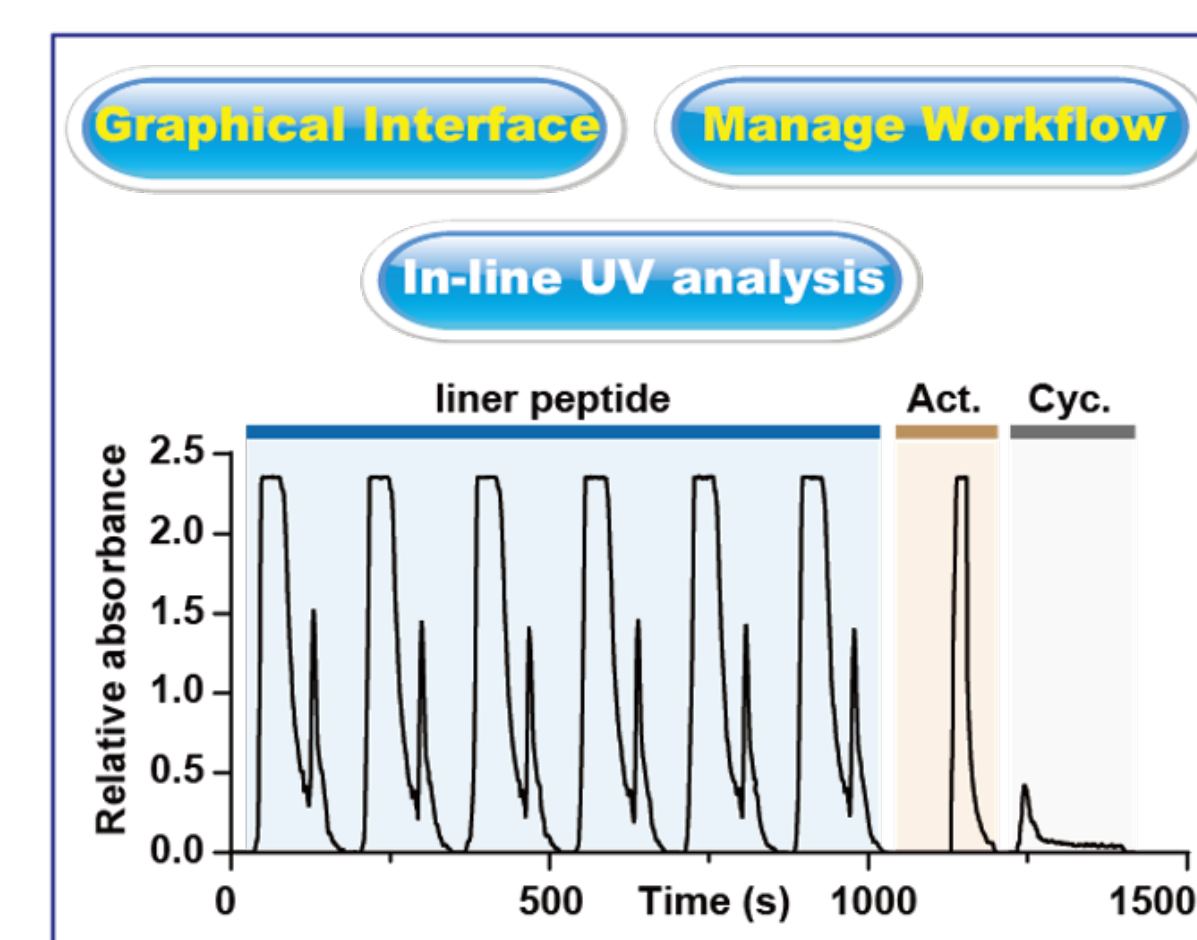
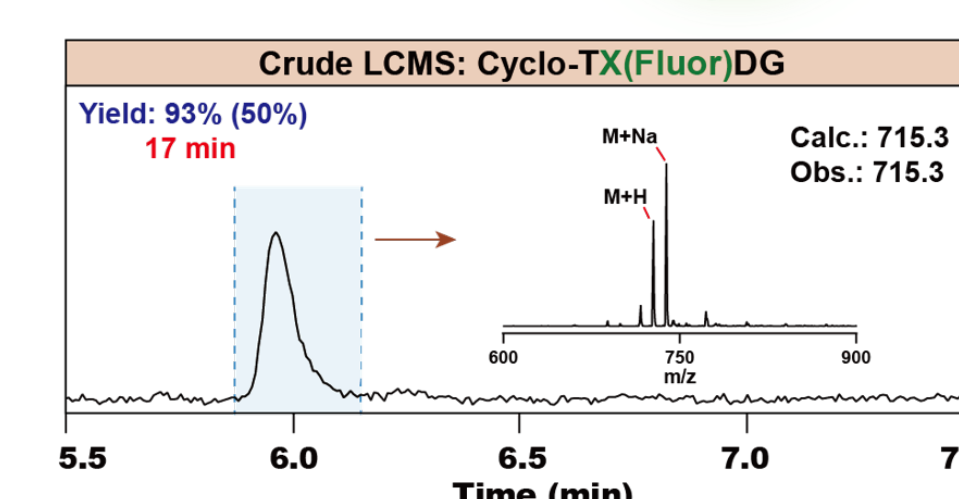
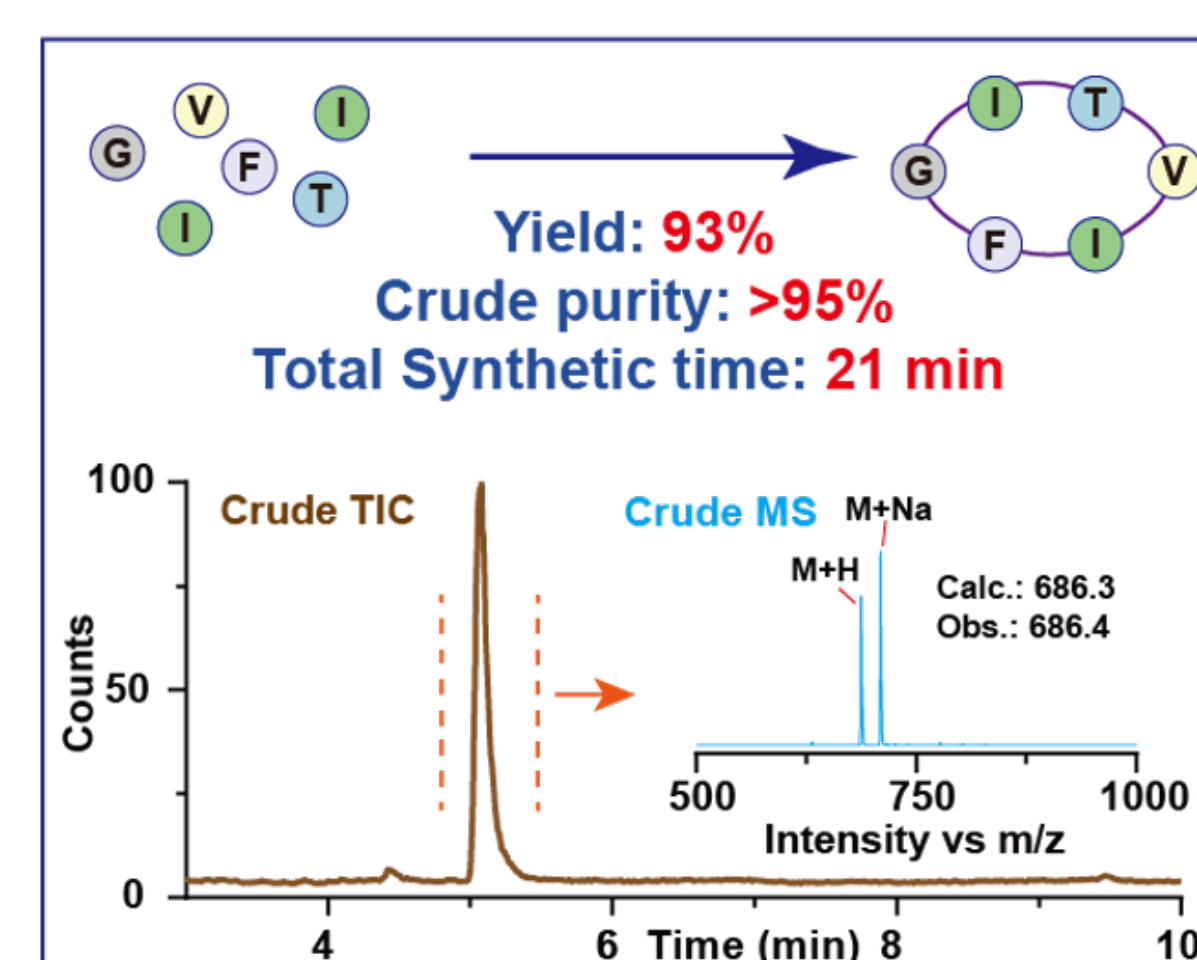
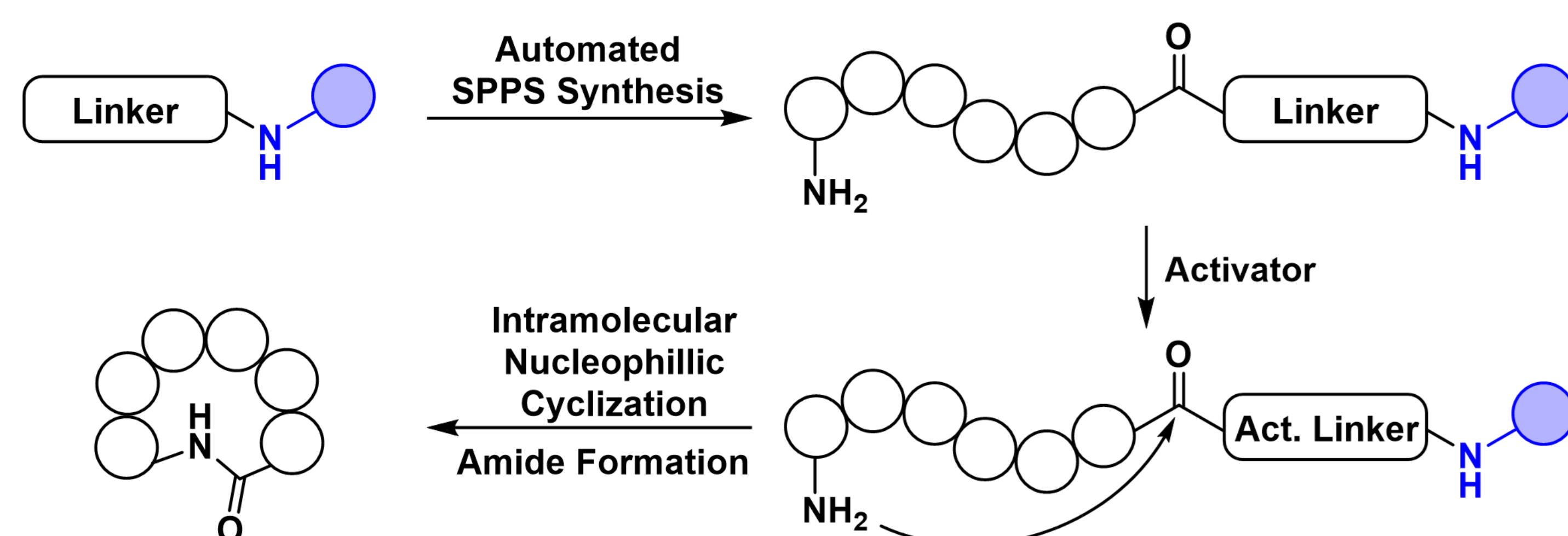
Introduction: This work reports the progress of a fully automated fast-flow synthesizer constructed with flexible configurations and a precise reaction control mechanism to match the specific needs of a variety of chemistries required for various synthesis applications. By using the automated platform, a greater range of applications, higher synthesis efficiency, and improved purity over some of the commercial devices on the market have been demonstrated. With flow-based synthesis, capping or double couplings may be eliminated. The integrated programmable temperature control design increases coupling efficiency while reducing on-resin aggregation and other side reactions.

Instrumentation: The all-in-one synthesizer device can be configured to install up to three piston pumps, more than 20 monomer inlets, more than 6 activation agent inlets, and more than 8 other reagent inlets. Installation options also include collection valves, a UV detector module, purge gas regulators, and other solid-phase reactor compartments.



Acknowledgement: Dr. Jack Zhou and NSP LLC collaborated on the design and development of the synthetic device along with its software control system. Dr. Wan Feng and Professor Li Chengxi developed a single-shot synthesis process for natural cyclic peptides and conducted experimental validation on TrueSynt.

Experiments: The diagram above shows the flow diagram of an automated fast-flow solid-phase synthesizer. It includes programmable temperature-controlled reagent delivery and reusable reactors. Monomers, activating agents, and other reagents are mixed using two or three high-precision pumps per chemistry involved. Rotary valves equipped with 20+ inlets are used to select the monomers, activating agents, and other reagents. A mixer, followed by a temperature-controlled mechanism, conditions the reactions to take place. Monomers, depending on their respective chemistry, are activated at 70°C or another target temperature in a loop, then flowed over the resin bed housed in a reusable reactor. The effluent is passed through a UV-vis spectrometer for collection or disposal.



Results: The validation of the synthesizer is used for the synthesis of linear peptides on our proprietary linker. After synthesis, the activation reagent interacts with the linker to generate an activated linker, allowing in situ cyclization on the TrueSynt for cyclic peptide synthesis. The entire cyclization process takes 8 minutes, resulting in a highly pure crude cyclopeptide. For example, Cyc-GITVIF synthesis took 21 minutes, with a crude purity of 95% and an NMR yield of 93%.