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Application of an Automated System for Flow Solid Phase Peptide Synthesis

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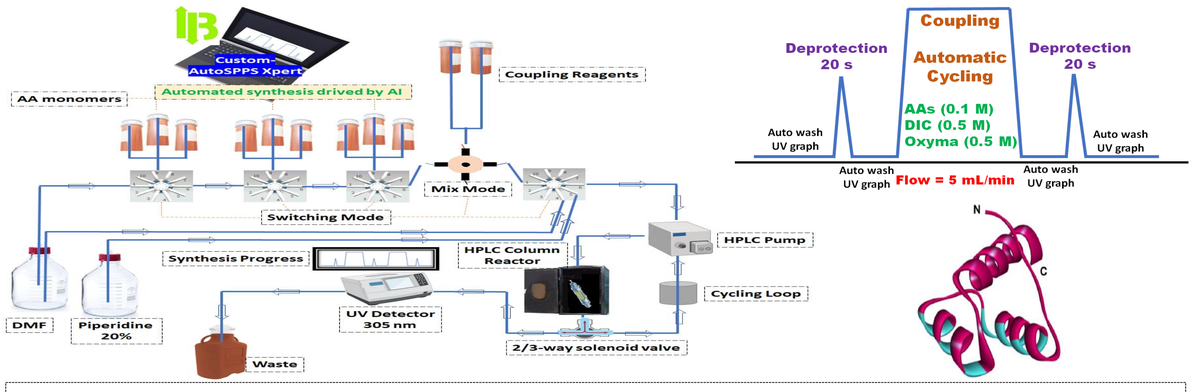
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Introduction

Over the past 60 years, significant advancements have been made in SPPS technologies, including flow-based SPPS.[1,2] In particular, the overall progress of batch SPPS is limited, and ligation chemistry for longer peptides/proteins is highly time-consuming, laborious, leading to undesired side-products, and relatively low yields. Nowadays, flow-based SPPS is gaining momentum due to its advantageous features—for example, control over physical parameters and greatly reduced formation of side products. Recently, it has been demonstrated that the automatic fast-flow peptide synthesis (AFPS) technology is highly effective, however, produces substantial amount of waste due to uses of high amount of reagents and DMF.[3] Therefore, a single-shot and effective SPPS for large and complex proteins with a low amount of waste production remains a challenge. Nowadays, the chemical synthesis of miniproteins/proteins has been of great interest due to their broad applications from basic research to the manufacture of clinically approved therapies, but it is far from being a fail-safe method for all.[2,4-7] To overcome all these challenges in SPPS, we describe the development of a custom-build fully automated flow SPPS technology and its optimizations for the synthesis of peptides, miniproteins, and proteins, yielding high-purity crude products with less production of waste.

Results and Discussion



Schematic Setup for Automatic Flow SPPS, Operation, and Studied Miniprotein (PDB ID 1ENH)

No.	Peptide	Coupling (AA/DIC/Oxyma)	Time of Deprotection (s)	Crude amount (mg)	Crude Purity
1	ENH	3/3/3	60	120	NA
2	ENH	4/4/4	40	132	10%
3	ENH	4/6/4	30	130	12%
4	ENH	4/8/4	25	134	11%
5	ENH	5/5/5	25	135	45%
6	ENH	6/6/6	20	142	70%
7	ENH	8/8/8	25 sec	139	45%
8	ENH	10/10/10	25 sec	153	42%

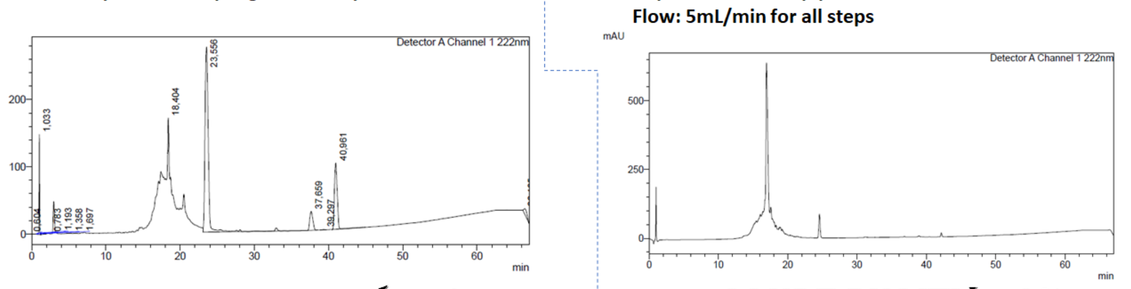
Optimization conditions for the Flow SPPS of ENH miniprotein

INITIAL CONDITIONS:

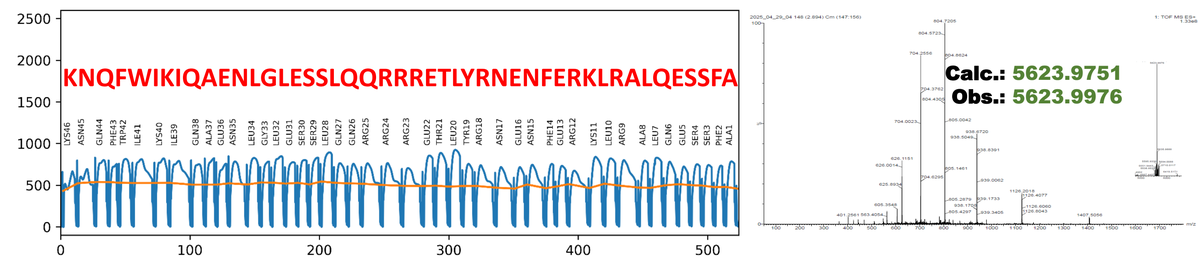
Temperature: 85-70 °C
Coupling step: 0.1 M amino acids stock solution in DMF
0.5 M oxyma stock solution in DMF
0.5 M DIC stock solution in DMF
Deprotection step: 20% piperidine solution in DMF
Flow: 1 mL/min for coupling and 2.5mL/min for wash

FINAL CONDITIONS:

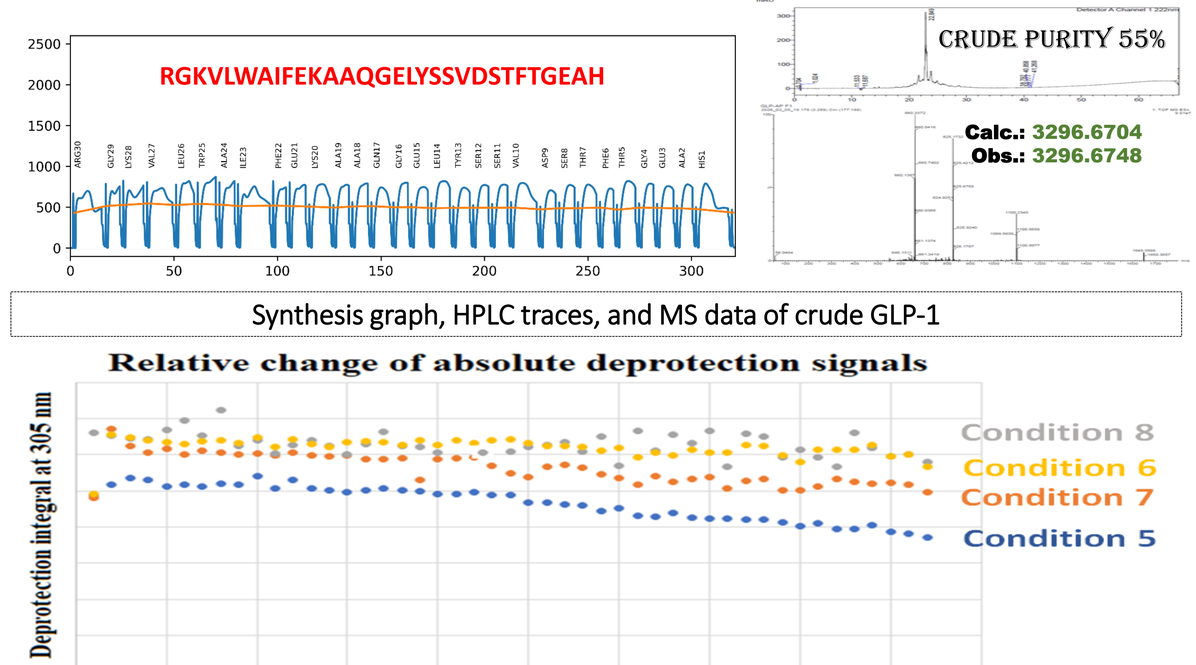
Temperature: 70 °C
Coupling step: 0.1 M amino acids stock solution in NMP
0.5 M oxyma stock solution in NMP
0.5 M DIC stock solution in NMP
Automatic Cycling fully AI based
Deprotection: 20% piperidine solution in DMF
Flow: 5mL/min for all steps



HPLC traces of ENH miniprotein before and after optimized conditions



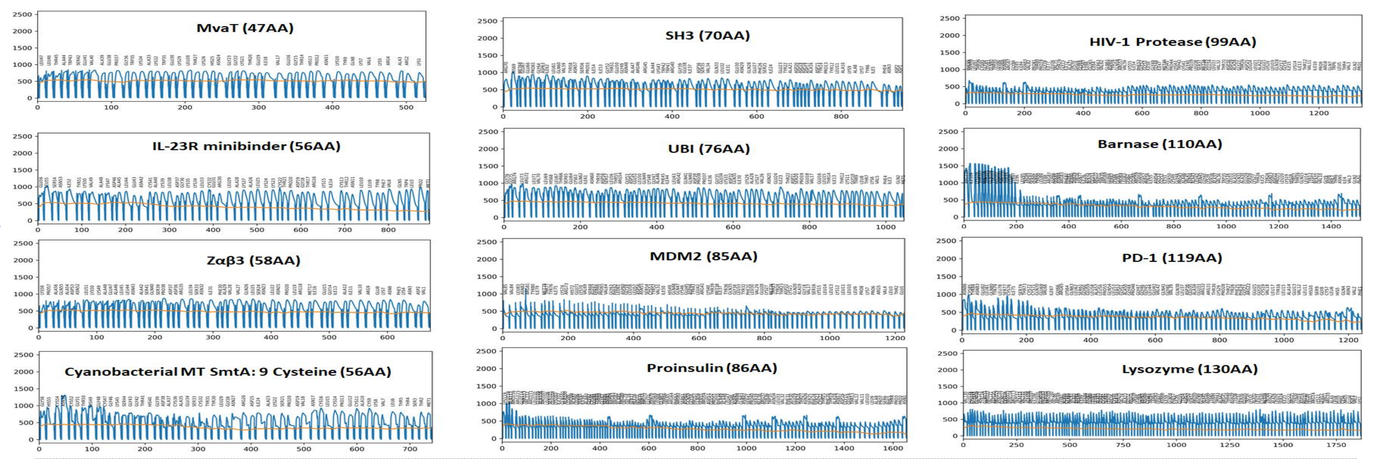
Synthesis graph and MS data of crude ENH miniprotein



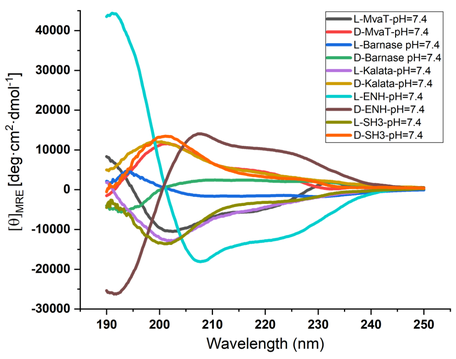
Synthesis graph, HPLC traces, and MS data of crude GLP-1

SPPS	Sequence	Length
EETII	GCPRILMRCKQDSDCLAGCVCGPNGFCG	28
NN92	RVVVGEHNLSQNDGTEQYVNVQKIVSHPY	29
Kalata B1	CGETCVGGTCNTPGCTCSWPVCTRNGLPV	29
D-Kalata B1	CGETCVGGTCNTPGCTCSWPVCTRNGLPV	29
GLP-1	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR	30
5JI4	APCECDVNGETYTVSSSEECERLCRKLGVTNCRVHCG	37
Amyloid β	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA	42
ENH	AFSSEQLARLKREFNENRYLTERRQQLSSELGLNEAQIKIWFQNK	46
D-ENH	AFSSEQLARLKREFNENRYLTERRQQLSSELGLNEAQIKIWFQNK	46
MvaT	KRARKVKQYKNPHTGEVIETKGGNHKTLKEWKAKWGPEAVESWATLL	47
D-MvaT	KRARKVKQYKNPHTGEVIETKGGNHKTLKEWKAKWGPEAVESWATLL	47
IL-23R	MPLWQVFYLLNTCIKRTGDPTCKKLAKALRECLKKGDLKACNELADKAVKYINSLE	56
MT SmtA	MTSTTLVKCACEPCLCNVDPSKAIDRNGLYYCSEACADGHTGGSKGCGHTGCNCHG	56
BPTI	RPAFCLEPPYAGPGKARIIRYFYNAAAGAAQAFVYGGVRAKRNNFASAADALAACAAA	58
Z(αβ3)	VDNKFNKERVIAIGEIMRLPNLNSLQVVAFINSLRDDPSQSANLLAEAKKLNDAQAPK	58
DHFR	VFPSNATFGMGDRVRKKSGAAWQGQIVGWYCTNLTPEGYAVESEAHPGSVQIYPVAALERIN	62
SH3	DDNFIYKAKALYPYDADDDDAYEISFEQNEILQVSDIEGRWWKARRANGETGIIPSNYVQLIDGPEEMHR	70
D-SH3	DDNFIYKAKALYPYDADDDDAYEISFEQNEILQVSDIEGRWWKARRANGETGIIPSNYVQLIDGPEEMHR	70
UBI	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNI QKESTLHLVLRLRGG	76
MYC[1-84]	MPLNVSFTNRNYDLDYDSVQPYFYCDEEENFYQQQQQSELQPPAPSEDIWKKFELLPTPPLSPSRRSGLCSPSYVAVTPFSLRG	84
MDM2	ETLVRPKPLLLKLLKSVGAQKDTYTMKEVLFYLGQYIMTKRLYDEKQQHIVYCSNDLLGDLFGVPSFSVKEHRKIYTMIYRNLVV	85
D-MDM2	ETLVRPKPLLLKLLKSVGAQKDTYTMKEVLFYLGQYIMTKRLYDEKQQHIVYCSNDLLGDLFGVPSFSVKEHRKIYTMIYRNLVV	85
Proinsulin	FVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAEDLQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCN	86
Barstar	KKAVINGEQIRSISDLHQTLKKELALPEYYGENLDALWDCLTGWVEYPLVLEWRQFEQSKQLTENGAESVLQVFREAKAEGCDITIILS	89
ERG	MEEKHMPPPNMTTNERRVIVPADPTLWSTDHVRQWLEWAVKEYGLPDVNILLFQNIDGKELCKMTKDDFQRLTPSYNADILLSHLHYLRETPLP	94
BCL11A	RSDTCEYCGKVFKNCSNLTVHRRSHTGERPYKCELCNYACAQSSKLTRHMKTHGQVGKDVYKCEICKMPFSVYSTLEKHMKKWHSDRVLNNDIKTE	96
HIV-Protease-1	PQVTLWQRPIVTIKIGGQLKEALLDTGADDTVLEEMSLPGKWKPKMIGGIGGFIKVRQYDQVSIEICGHKAIGTVLIGPTPVNIIGRNLLTQLGCTLNF	99
HIV-Kent type	PQITLWKRPLVTIRIGGQLKEALLDTGADDTVIEEXNLPGKWKPKXIGGIGGFIKVRQYDQIPVEIZGHKAIGTVLVGPTPVNIIGRNLLTQIGATLNF X = norleucine, Z = aminobutyric acid	99
Collagen	GLPGAKGLAGAPGAPGPDZKAGPPGPAGQDCpsRPGPPGPPGARGQAGPPGFPGPKGAAGEPGKAGERGVPGPPGAVGPAGKDCpsEAGAQG PPGPAGPAGER Z = Fmoc-(Dmb)Gly-OH building blocks)	99
TRX-1	SDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQL KEFLDANLA	108
Barnase	AQVINTFDGVADYLQTYHKLPDNYITKSEAQALGWVASKGNLADVAPGKSIGGDIFSNREGKLPGKSGRTWREADINYTSGFRNSDRILYSSDWLIY KTTDHYQTFTKIR	110
D-Barnase	AQVINTFDGVADYLQTYHKLPDNYITKSEAQALGWVASKGNLADVAPGKSIGGDIFSNREGKLPGKSGRTWREADINYTSGFRNSDRILYSSDWLIY KTTDHYQTFTKIR	110
Barnase[R110FBr]	AQVINTFDGVADYLQTYHKLPDNYITKSEAQALGWVASKGNLADVAPGKSIGGDIFSNREGKLPGKSGRTWREADINYTSGFRNSDRILYSSDWLIY KTTDHYQTFTKIX X = (p-Br-Phe)	110
PD-L1[18-133]	AFTVTVPKDLYVVEYGSNXTIECKFPVEKQLDLAALIVYWEXEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCX ISYGGADYKRITVKVNAPK X= norleucine	117
STIP1	MEQVNELKEKGNKALSVGNIDDALQCYSEAIKLDPHNHVLYSNRSAAYAKKGDYQKAYEDGCKTVDLKPDWGKGYSRKAAALEFLNRFEEAKRTYE EGLKHEANNPQLKEGLQNMEAR	118
PD-1[33-150]	NPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRXSPSNQTDKLAAFPEDRSQPGQDSRFRVTQLPNGRDFHXSVVRARRNDSGTYLCGAISLA PKAQIKESLRAELRVTERRAEK X= norleucine	119
Ribonuclease A	KETAAAKFERQHMDSSTSAASSSNYCNQMMKSRNLTKDRCKPVNTFVHESLADVQAVCSQKNVACKNGQTNCYQSYSTMSITDCRETGSSKYPN CAYKTTQANKHIIVACEGNPYVPVHFDASV	124
MDM2[1-118]	MHHHHHHGSMCNTNMSVPTDGAVTTSQIPASEQETLVRPKPLLLKLLKSVGAQKDTYTMKEVLFYLGQYIMTKRLYDEKQQHIVYCSNDL LGDLFGVPSFSVKEHRKIYTMIYRNLVVVNQQESSDS	127
Lysozyme	VFERCELARTLKRLGMDGYRGISLANWMCLAKWESGYNTRATNYNAGDRSTDYGIFQINSRYWCNDGKTPGAVNACHLSCSALLQDNIA DAVACAKRVVRDPQGIRAWVAWRNRCQNRDVRQYVQGCGV	130

List of AI- supported cost-effective flow-SPPS of proteins in this work



Some Examples of Protein Syntheses With Our AI-Model Approach



CD-spectra measurement for L- and D-conformation of selected proteins

Conclusions

- AI-supported efficient fully automated flow technology has been developed for SPPS.
- The initial optimization of synthesis parameters such as flow, pressure, temperature, and duration of individual steps leads to a AI-supported methodology that allows peptides and miniproteins/proteins synthesis up to 100 residues in a routine manner.
- The low usage of solvents and reagents provided the high purity crude products is another advantage of this approach. ***Our AI-supported flow SPPS technology also provides the access to D-conformation proteins with high crude purity validated by MS, analytical HPLC, and CD results.***

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