

## ABSTRACT

Typical robotic peptide library synthesizers run the same program in all reaction vessels throughout a synthesis. Because of this, if special programs are required to synthesize a modified peptide, those modifications must occur in the same cycle for all of the peptides. Also, it is not possible to synthesize peptides of different lengths in the same synthesis. The Overture™ Robotic Peptide Library Synthesizer was designed to overcome these shortcomings of existing robotic units. In this poster, we demonstrate the synthesis of different length peptides with modifications in different positions all in the same synthesis on the Overture™.

## INTRODUCTION

In nature, post translational modifications such as methylation, acetylation, phosphorylation and ubiquitination can affect protein function [1]. In the case of histone proteins (which are associated with DNA in chromatin), post translational modifications can affect gene expression as well as other DNA-templated processes in the cell [1]. In addition to the modifications found in nature, synthetic peptides often include modifications such as biotinylation, pegylation, and fluorescent labels for use in biological assays.

Typically, robotic peptide library synthesizers must run the same program in all reaction vessels during a synthesis. If special programs are necessary to synthesize a modified peptide, only peptides with modifications in the same position can be synthesized at the same time. It is also not possible to efficiently synthesize peptides of different lengths in the same synthesis. The Overture™ Robotic Peptide Library Synthesizer (Figure 1) was designed to overcome these shortcomings, and can easily synthesize different length peptides with modifications in different positions all in the same synthesis.

To illustrate this, four histone peptides of varying lengths (Figure 2) were synthesized on a 2x2 reaction vessel block on the Overture™. Modifications included biotinylation, pegylation, and acetylation, which occurred in different positions within the sequences. Sequences were selected from a combinatorial library of 110 modified histone peptides originally synthesized by Dr. Brian Strahl's laboratory at the University of North Carolina School of Medicine [1].



**Figure 1:** The Overture™ Robotic Peptide Library Synthesizer from Protein Technologies, Inc.

66: Ac-SGRGK(Ac)GGKGLGKGGAKRHRKVL-PEG-Biotin  
70: Ac-SGRGK(Ac)GGKGLGK(Ac)GGAKRHRKVL-PEG-Biotin  
72: Ac-SGRGK(Ac)GGK(Ac)GLGK(Ac)GGAKRHRKVL-PEG-Biotin  
301: Ac-SGRGK(Ac)QGK(Ac)ARAK(Ac)AK(Ac)TR-PEG-Biotin

**Figure 2:** Modified histone peptide sequences synthesized on the Overture™. The numbers to the left correspond to the sequence labels on Dr. Brian Strahl's website [2].

## EXPERIMENTAL

**Materials:** Biotin-PEG NovaTag resin and Fmoc-Lys(Ac)-OH were purchased from Novabiochem (Boston, MA). TFA, HATU, NMM and solvents were provided by Protein Technologies, Inc. (Tucson, AZ).

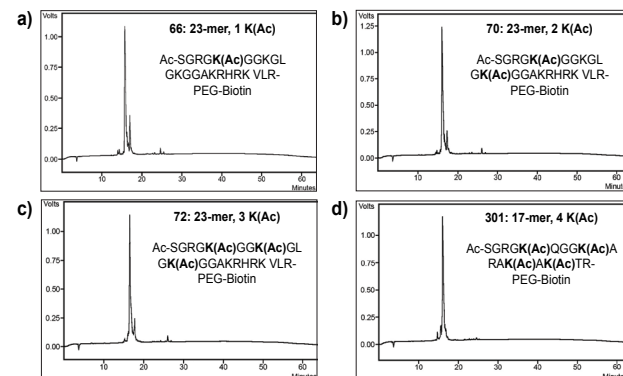
**Synthesis:** The peptides were synthesized at the 20 μmol scale on an Overture™ Robotic Peptide Library Synthesizer on Biotin-PEG NovaTag resin (0.47 mmol/g substitution). Deprotection: 20% piperidine/DMF 2 x 7 min. Coupling: 1:1:2 0.1M AA/0.1M HATU/0.2M NMM/DMF (10x excess) for 50 min (standard amino acids), or for 2 x 50 minutes (Fmoc-Lys(Ac)-OH couplings). N-terminal acetylation was performed by treating the resin with 1:1:3 acetic anhydride/NMM/DMF for 30 minutes following the final deprotection. Cleavage: 95/2.5/2.5 TFA/TIS/water for 2 hours.

**Analysis:** Crude peptides were precipitated in ice cold ether, dissolved in water and analyzed on a Varian ProStar HPLC using a C18, 300 Å, 5 μm, 250 x 4.6 mm column (Varian Microsorb-MV), over 60 minutes with a flow rate of 1 mL/min, and using a gradient of 5-95% B, where Buffer A was 0.1% TFA in water, and Buffer B was 0.1% TFA in acetonitrile. Detection was at 214 nm. Mass analysis was performed on a Shimadzu

LCMS-2020 Single-Quad mass spectrometer, equipped with a C18, 100 Å, 2.6 μm, 50 x 2.1 mm column (Phenomenex Kinetex), over 7 minutes with a flow rate of 1 mL/min and using a gradient of 5-50% B where Buffer A was 0.1% formic acid in water and Buffer B was 0.1% formic acid in acetonitrile.

## RESULTS

Four modified histone peptides were synthesized on the Overture™ in a single synthesis on a single 2x2 block. The peptides ranged in length from 17 to 23 amino acids, and contained 1, 2, 3, or 4 modified lysine residues, which were coupled under different conditions than the standard amino acids. Mass analysis verified the main peak was the product peptide in each case, allowing for easy purification (data not shown). HPLC data is shown in Figure 3.



**Figure 3:** HPLC results for crude peptides.

## CONCLUSIONS

Unlike existing robotic platforms, the Overture™ is an extremely flexible platform for synthesizing modified peptides. Peptides of different lengths with modifications in different positions can be successfully synthesized in one synthesis.

## ACKNOWLEDGEMENTS

Special thanks to Dr. Brian Strahl of the University of North Carolina School of Medicine for the sequences and many useful discussions.

## REFERENCES

[1] Fuchs SM, Krajewski K, Baker RW, Miller VL, Strahl BD. Influence of Combinatorial Histone Modifications on Antibody and Effector Protein Recognition. *Curr Biol* 2011; 21: 53-58.

[2] Online Analytical Data: [http://www.med.unc.edu/~bstrahl/Arrays/Pep\\_t.htm](http://www.med.unc.edu/~bstrahl/Arrays/Pep_t.htm)