Comprehensive Peptide Microarrays for Histone Research

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Introduction

Histones are important regulators of key processes including DNA replication, transcription, and DNA repair in healthy and diseased cells. Their regulation is mainly based on posttranslational modifications (PTMs), such as methylation, acetylation, phosphorylation, etc. Despite their importance, studying the complex dynamic effects of histone modifications is difficult because of the high number of potential PTM sites and sequence variants, as well as new PTMs that are continuously identified.

High-density peptide microarrays are ideal tools for the parallel presentation and examination of peptides. Their value has been proven in numerous applications, e.g. the investigation of humoral immune responses, biomarker discovery, enzyme/antibody profiling and the identification of enzyme substrates.

We developed a peptide library representing almost 4000 peptides with all potential PTMs in Histones H1, H2A, H2B, H3 and H4, including all available sequence variants from protein databases. The PTMs include phosphorylation, methylations, acetylation, citrullination, malonylation, succinylation, butyrylation and propionylation. This peptide microarray platform enables the characterization of binding events and interactions with modified histone peptides in unprecedented detail.

Experimental Design

Peptide library design (20mers)

- **Knowledge Based Library: 1300 Peptides**

  Histone H2A, H2B, H3, H4 with PTMs at all reported sites ("KBut", "KAc", "KProp", "KMe1", "KMe2", "KMe3", "RMe1", "RMe2a", "RMe2s", "Cit“, "pT", "pS", "pY"); known PTM combinations (up to 6 PTMs); representation of natural sequence variants; H1 wildtype scan plus modifications as published in Wisniewski et al. *Mol. Cell Proteomics.* **2007**, *6(1)*, 72-87

- **Systematic Library: 2400 Peptides**

  Limited scan through histones H2A, H2B, H3, H4 with single modifications (PTMs at all potential sites)

Peptides are printed on glass slides resulting in the Histone Code Array (HCA)

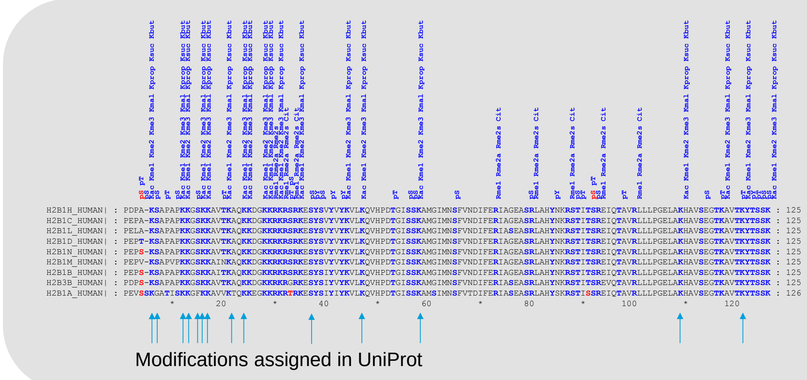

Fig. 1 Sequence variants shown exemplarily for H2B, phosphorylation, 4 Arg modifications, 8 Lys modifications.
570 peptides cover complete diversity and all single modifications (instead of 1189 protein variants).

Results and Conclusions

Histone Code Arrays (HCA) can be used for various applications like epitope mapping of PTM specific antibodies, investigation of substrate specificity of PTM-enzymes (acetylases, deacetylases etc.) and investigation of binding specificity of histone binding proteins and protein domains (e.g. Bromo domains). In Fig. 2 and 3 two examples are shown for the first two applications.

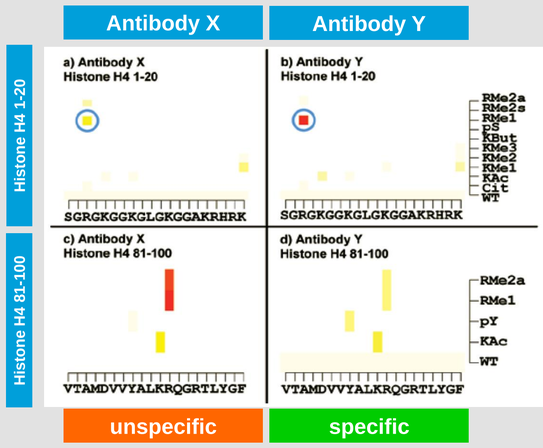

Fig. 2 Specificty test for two different H4R3me1 antibodies.

➡ Antibody Y shows strong signal for H4R3me1 without significant cross-reactivity, whereas antibody X preferably recognizes H4R29me1

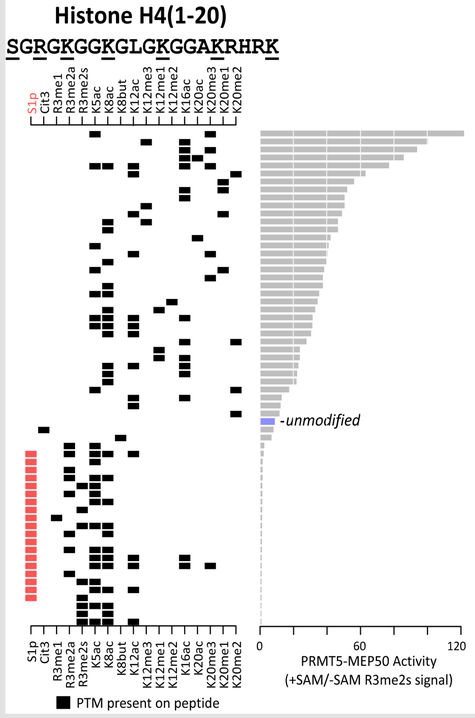

Fig. 3 Substrate specificity of histone modifying enzymes. As example, PRMT5-MEP50 was incubated +/- SAM on HCA. A H4R3me2 specific antibody was subsequently used to monitor the methylation events.

➡ Phosphorylation of Serine 1 inhibits the methylation mediated by PRMT5-MEP50

The Histone Code Peptide Array (HCA) provides

- **A tool for studying histone interactions with unprecedented detailedness**
- **Broad collection of peptides addressing diversity of isoforms and modifications**
- **Three identical subarrays for improved data quality**
- **Readout in a single experiment with very low consumption of sample**
- **Availability of high quality peptide sets allows fast and efficient confirmation of results (e.g. Histone Code Peptide ELISA)**

References

[1] Ho MC, Wilczek C, Bonanno JB, Xing L, Seznec J, Matsui T, Carter LG, Onikubo T, Kumar PR, Chan MK, Brenowitz M, Cheng RH, Reimer U, Almo SC, Shechter D, PLoS One (2013) Structure of the arginine methyltransferase PRMT5-MEP50 reveals a mechanism for substrate specificity.

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