

## Use of High-Density Histone Peptide Arrays for Parsing the Specificity of a Histone-Modifying Enzyme Complex

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Methylation of arginine residues by protein arginine methyltransferases (PRMTs) is an underexplored component of the histone code of epigenetic regulation. The histone code encompasses an exceedingly complex combination of post-translational modifications (PTMs) of histone residues that marks and directs utilization of the underlying genome. We created a peptide array containing all core, variant and linker histone primary amino acid sequences and all known and possible post-translational modifications, alone and in combination. Here, we utilized these arrays to study the substrate specificity of a critical enzyme complex.

### Introduction

Chromatin - the compacted higher-order structure of DNA packaged with proteins - is the physiological form of the eukaryotic genome (7). The fundamental unit of chromatin is the nucleosome, in which DNA is wrapped around an octamer of the core histones, H2A, H2B, H3, and H4 (6). Post-translational modification (PTM) of histones, such as arginine methylation, forms a “histone code” regulating the usage of the underlying DNA (8). Arginine methylation of histones is carried out by a family of protein arginine methyltransferases (PRMTs) that have diverse roles (3, 4). The majority of these enzymes are Type I enzymes capable of mono- and asymmetric-dimethylation of arginine (annotated as Rme1 and Rme2a) (1). PRMT5 is the major Type II enzyme in eukarya, capable of mono- and symmetric-dimethylation of arginine (Rme1 and Rme2s). PRMT5 is always found complexed with the WD-repeat protein MEP50. PRMT5 and MEP50 are both required for embryogenesis and misregulated in a variety of cancers. Strikingly little is known about arginine methyltransferase activity, specificity, and post-translational modification cross-talk on activity.

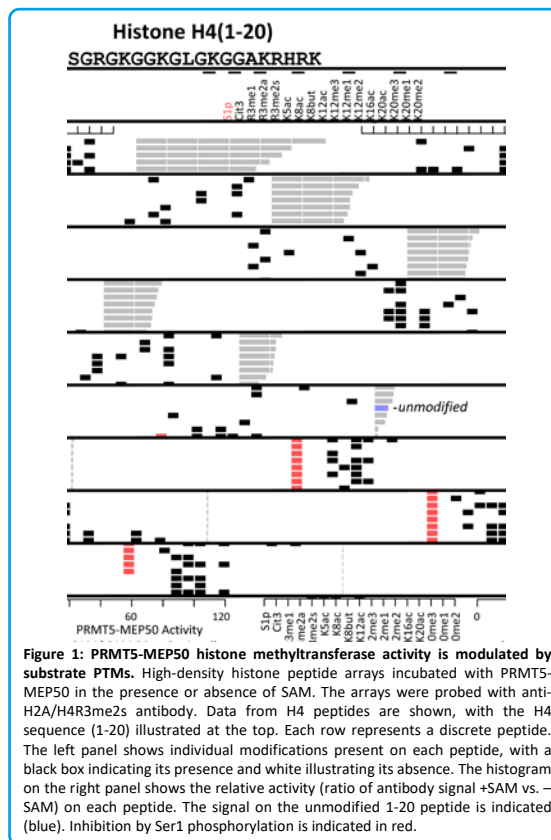
We recently solved the crystal structure of the *Xenopus laevis* PRMT5-MEP50 complex (5). To test the substrate specificity and mechanism of substrate recognition used by the enzyme complex we utilized the high-density histone peptide arrays produced by JPT Peptide Technologies (Berlin, Germany) containing more than 3800 human core histone peptides. These peptides covered all of the primary amino acid sequence of core histones and variants, as well as all known and hypothesized PTMs, alone and in combination. These studies with PRMT5-MEP50 demonstrated the striking sensitivity of this histone modifying enzyme’s activity to neighboring post-translational modifications. Using these arrays we also showed that the complex primarily bound to histones through histone fold and C-terminal domains, and that these interactions were also sensitive to post-translational modifications on the N-terminal tail. The complete sequence coverage across core histones and histone variants as well as the presence of common and rare histone posttranslational modifications – alone and in combination – made these arrays an unparalleled tool for studying the writing of the histone code.

### Results

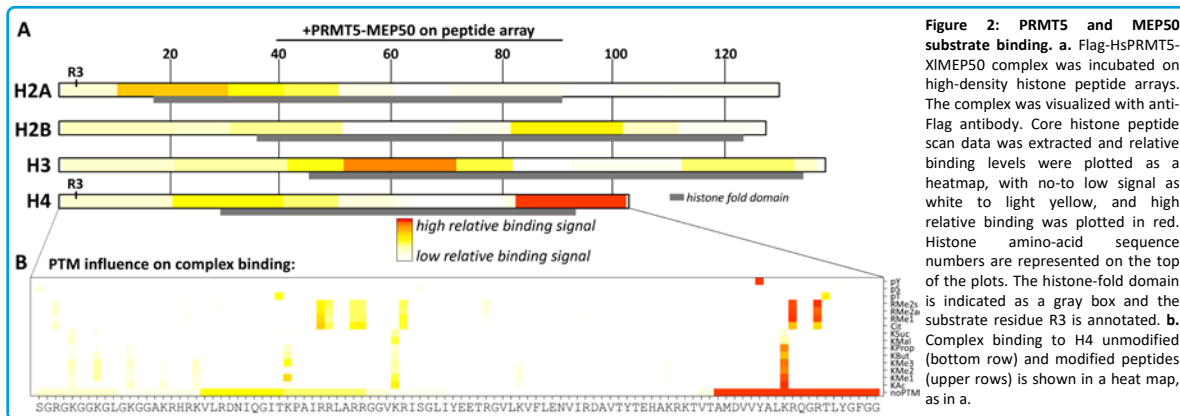
Since H2A and H4 Arg3 are known PRMT5-MEP50 substrates, we developed an H2A/H4 R3me2s antibody based readout to assay its activity on the histone code peptide arrays. By definition this limited the activity determination to those peptides recognized by the antibody. The highly specific readout of the antibody on the array for histone H4 peptides containing R3 symmetric dimethylation is shown in Supplemental Figure S5 in reference (5). Neighboring PTMs modestly altered the signal, but still permitted readout. Antibody based assays are the best approach for activity tests on the array as they permit sensitive fluorescent detection

via secondary antibodies. Future production of “clickable” S-adenosylmethionine (SAM) analogs, the methyl-donor in the reaction, will permit direct detection of methylation regardless of the targeted residue and will be exceptionally useful for further development of this technology (2, 9).

We then incubated the PRMT5-MEP50 complex on the array in the absence and presence of SAM. Incubation of the treated array with the anti-R3me2s antibody followed by fluorescence secondary antibody and detection allowed us to determine the PTM code that influenced PRMT5-MEP50’s methyltransferase activity (plotted as the ratio of signal in the presence of SAM/absence of SAM). As shown in **Figure 1**, with data extracted from the H4 peptides on the array, Serine-1 phosphorylation completely eliminated activity while various combinations of lysine acetylation and lysine methylation on the histone tail peptide enhanced PRMT5-MEP50 activity.



**Figure 1: PRMT5-MEP50 histone methyltransferase activity is modulated by substrate PTMs.** High-density histone peptide arrays incubated with PRMT5-MEP50 in the presence or absence of SAM. The arrays were probed with anti-H2A/H4R3me2s antibody. Data from H4 peptides are shown, with the H4 sequence (1-20) illustrated at the top. Each row represents a discrete peptide. The left panel shows individual modifications present on each peptide, with a black box indicating its presence and white illustrating its absence. The histogram on the right panel shows the relative activity (ratio of antibody signal +SAM vs. -SAM) on each peptide. The signal on the unmodified 1-20 peptide is indicated (blue). Inhibition by Ser1 phosphorylation is indicated in red.



**Figure 2: PRMT5 and MEP50 substrate binding.** a. Flag-HsPRMT5-XIMEP50 complex was incubated on high-density histone peptide arrays. The complex was visualized with anti-Flag antibody. Core histone peptide scan data was extracted and relative binding levels were plotted as a heatmap, with no-to low signal as white to light yellow, and high relative binding was plotted in red. Histone amino-acid sequence numbers are represented on the top of the plots. The histone-fold domain is indicated as a gray box and the substrate residue R3 is annotated. b. Complex binding to H4 unmodified (bottom row) and modified peptides (upper rows) is shown in a heatmap, as in a.

To determine how PRMT5-MEP50 recognizes substrate, we utilized the histone peptide array to characterize the complex's interaction with histones. Since the array contains the entire sequence of the core histones and all the potential modifications, this approach allowed us to determine the primary binding sites of PRMT5-MEP50 on the histones and the influence of the PTMs on this binding. We incubated the Flag-tagged complex on the array and then detected it with anti-Flag antibody and a fluorescent secondary antibody. We mapped the major interactions on the unmodified histones to a schematic of the core histones, with the histone fold domains annotated in gray (Figure 2a). We also illustrated the influence of histone H4 PTMs on complex binding (Figure 2b). Pronounced loss of H4 C-terminal tail binding by PRMT5-MEP50 was observed for Y98ph, for instance. While this modification has not been observed *in vitro*, followup mass spectrometry and targeted bindings studies can specifically analyze this modification, among others.

**Conclusions**

Here we presented the use of JPT's new extremely high density histone peptide arrays for studies of the PRMT5-MEP50 complex substrate specificity. We used the array to determine the influence of histone substrate PTMs on the enzyme complex's activity and demonstrated that neighboring Ser1 phospho-rylation completely abrogated its activity, which we independently confirmed in solution assays (5). We also observed stimulation of activity by neighboring lysine acetylation and methylation. This combinatorial "writing" code will be exceptionally informative for future studies of histone modifications and highlight the unique and novel contributions of histone peptide studies. We also utilized these arrays for binding studies of the enzyme complex to its substrate. These assays uncovered a novel potential interaction surface on the histone fold, consistent with PRMT5-MEP50's inability to methylate nucleosomes, in which the histone fold is obscured by DNA. These novel observations on the peptide arrays will lead to future studies and novel discoveries.

**References**

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