dCypher™ Platform Reveals Novel and Disease-Relevant Chromatin Interactions

Nucleosomes are the physiological substrates of chromatin associated proteins (CAPs), including chromatin readers, writers, and erasers. Recent studies found that CAPs can engage multiple chromatin features, such as histone post-translational modifications (PTMs), nucleosomal DNA, or the nucleosome acidic patch. These results demonstrate the central role of the nucleosome context in defining CAP interactions, a context that is impossible to capture in traditional histone peptide-based assays.

EpiCypher developed the dCypher™ platform using defined nucleosome substrates to enable rapid and quantitative characterization of CAPs in their physiological context. This versatile technology can be applied to discovery binding assays, inhibitor screening and more.

Advantages

- Unprecedented diversity in a physiological context: >100 modified nucleosomes
- Improved signal & sensitivity vs. peptide arrays
- Lower protein input vs. arrays (nM vs µM)
- Compatible with full-length proteins and domains
- No prior knowledge of target PTM required
- HTS-compatible for inhibitor profiling

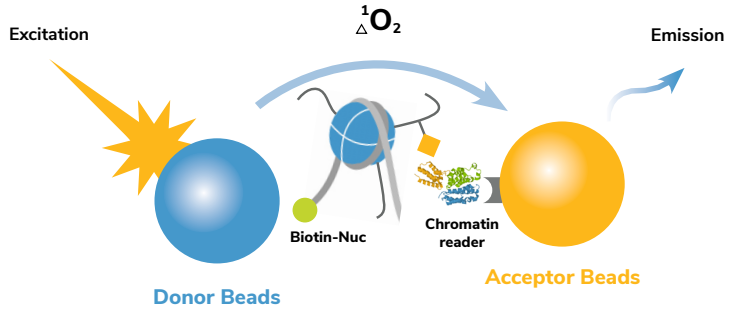


FIGURE 1 ***Nucleosome context used to interrogate chromatin associated proteins (CAPs).*** *The dCypher platform uses a luminescent, bead-based proximity assay to quantify nucleosome – CAP interactions.*

A powerful approach to study epigenetic crosstalk signaling

Epigenetic regulation of gene expression relies on crosstalk between histone PTMs and DNA methylation, an important phenomenon in human disease. For instance, mutations in either the H3K36 writer NSD1 or the DNA methyltransferase DNMT3A result in human growth disorders with similar phenotypes. However, the precise mechanisms linking these pathways remain poorly understood. As part of a recent collaboration, we applied dCypher assays to interrogate DNMT3A binding, revealing two competing histone PTM-based mechanisms that modulate DNMT3A recruitment[1].

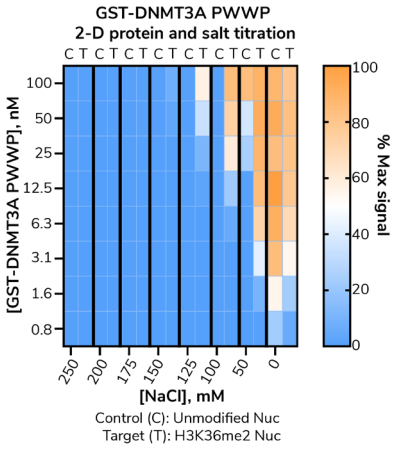


FIGURE 2A

dCypher assays conditions can be rapidly optimized. *Salt and protein concentration are key factors in nucleosome-based binding assays, and must be carefully selected for every system. Here, we performed a two-dimensional salt and protein titration to identify optimal binding conditions for the DNMT3A PWWP domain.*

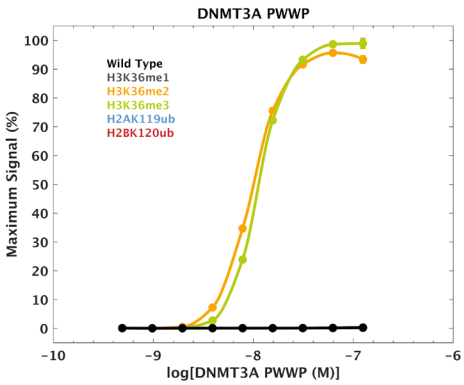


FIGURE 2B

Interrogate your protein against multiple nucleosome substrates in a single experiment using the dCypher platform. *Here, we titrated the DNMT3A PWWP domain against modified nucleosomes (representative set; potential of >100 nucleosome screening), revealing a strong preference for H3K36me2 and H3K36me3. This finding was further validated by ChIP-seq data, showing that DNMT3A co-localized with H3K36me2 / me3 at intergenic regions in vivo[1].*

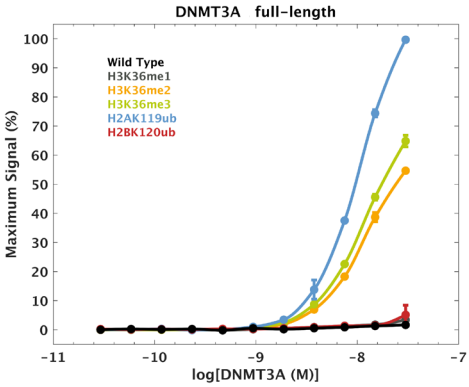


FIGURE 2C

dCypher assays are compatible with full-length (FL) proteins and reveal novel biology. *Here we interrogated the binding specificity of FL-DNMT3A against modified nucleosomes. We observed strong and selective binding to H2AK119ub, that was not present in PWWP assays, highlighting the role of a newly identified ubiquitin binding region in FL-DNMT3A[2].*

EpiCypher®
Bringing Epigenetics to Life

Validate novel chemical probes for drug development studies

The H3K36 writer enzyme NSD2 is aberrantly expressed, amplified, or mutated in multiple cancers, making it an excellent drug target. These efforts require potent chemical probes to identify druggable mechanisms, as well as nucleosome-based assays to define chromatin interactions. EpiCypher collaborated with the Structural Genomics Consortium to characterize a novel NSD2-PWWP1 domain chemical probe in dCypher assays (Figure 3)[3].

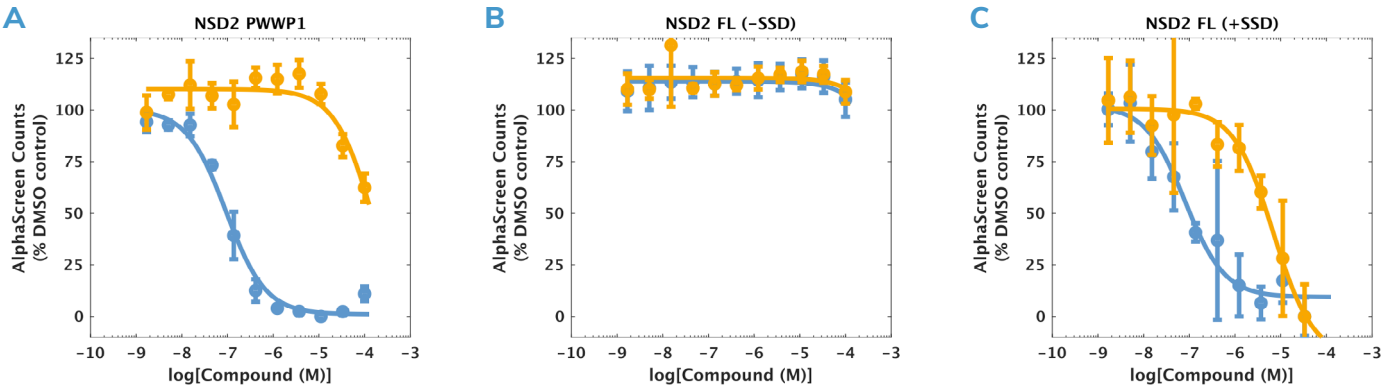


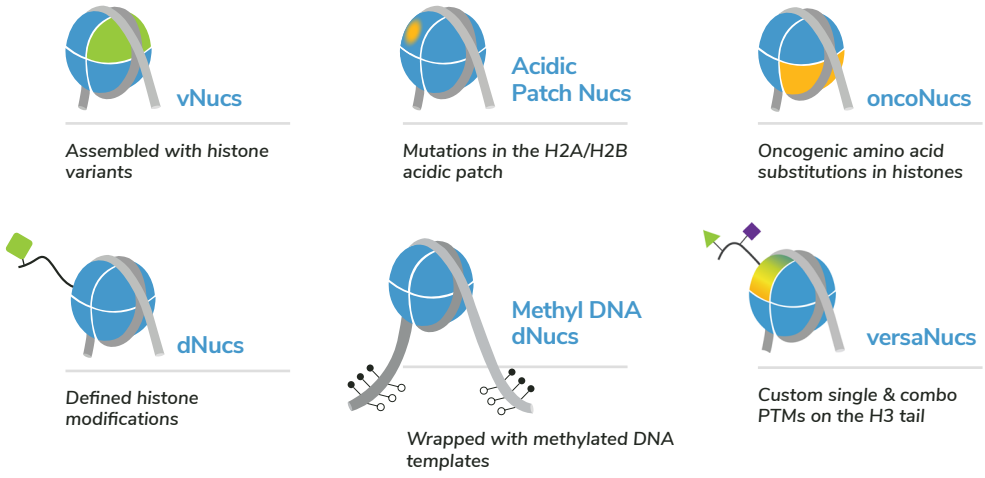
FIGURE 3 ***Optimization of dCypher assay reveals novel NSD2-PWWP1 chemical probe binding.*** *The NSD2-PWWP1 probe (UNC6934; blue) or negative control (UNC7145; orange) was titrated in a dCypher assay that quantified NSD2-PWWP1 binding to H3K36me3 nucleosomes. (A) UNC6934 blocks PWWP1 binding. (B) Neither probe disrupted full-length NSD2 binding under standard conditions. (C) Introduction of of exogenous salmon sperm DNA (SSD) rescued inhibitory activity against full length NSD3*[3].

dCypher™ Assay Services

Take the guesswork out of your chromatin binding assays. EpiCypher has considerable experience in developing and optimizing binding assays for CAPs, including readers, writer / eraser enzymes, and antibodies. Our expert scientists will optimize every aspect of your assay and screen your protein-of-interest using our expansive nucleosome library of over 100 modified nucleosomes, including a variety of histone modifications (dNucs), variant histones, mutants, and methylated DNA.

Sample Applications

- Decipher chromatin regulatory pathways
- Drug discovery pipelines
- Enzymatic assays
- Protein interaction studies
- Structural studies
- Assess antibody performance
- Or customize your own



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Let's discuss your project! Design your experiment with our expert scientists and leverage dCypher assays to reveal the true binding specificity of your protein!

Nucleosome Panels

Full Panel	16-9001
K-MetStat & OncoStat Panel	16-9002
K-AcylStat Panel	16-9003
R-MetStat Panel	16-9004

Citations

(1) Weinberg, DN, et al. Nature (2019)
(2) Weinberg, DN, et al. Nature Genetics (2021)
(3) Dilworth, D, et al. Nature Chemical Biology (2021 – in press)

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