dCypher™ Screening of BPTF Reader Domains Show Nucleosomes, Not Peptides, Dictate the Histone Code

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BACKGROUND

Epigenetic regulators recognize histone post-translational modifications (PTMs) through evolutionarily conserved binding domains (aka. readers), thereby recruiting nuclear complexes to specific genomic loci. Modified histone peptides are frequently used to model chromatin and enable characterization of [reader - histone PTM] specificity, and in doing so, decipher the histone code; however, peptides are structurally very different from chromatin and their use assumes PTM specificity is unaffected by higher order factors. To assess the limitations and shortcomings of this reductive approach, EpiCypher® has developed the dCypher assay, a high-throughput discovery platform for the rapid screening & detailed interrogation of chromatin interactors (readers, enzymes, and antibodies) against comprehensive libraries of modified histone peptides and designer nucleosomes. Here, we used dCypher assays to show PTMs presented on peptides engage individually with a dual PHD-BD domain of BPTF (subunit of the Nucleosome Remodeling Factor [NURF] complex); however, when the same modifications are presented on nucleosomes, combinatorial engagement drives a highly specific interaction. Further, genomic mapping with CUT&RUN recapitulates the specificity of the nucleosome-reader interaction, where reader-mediated enrichment is only seen when both methyl and acetyl marks are present. These findings demonstrate the critical importance of using nucleosome substrates to garner accurate insights into *in vivo* binding mechanisms.

dCypher Binding Assay

- Robust signal / background
  - Identifies hits missed by peptide arrays
- Highly sensitive no-wash format
  - Lower protein input requirements vs peptide arrays
- Accommodates full length & multidomain proteins
  - Assess cooperative binding
- Access comprehensive libraries
  - >287 modified histone peptides
  - >100 recombinant nucleosomes
- Unprecedented epigenetic diversity
  - PTMs: Kme, Rme, Kac, S/T/Y-phos
  - Other: DNAme, histone variants, onco-mutations
- Custom services by expert scientists

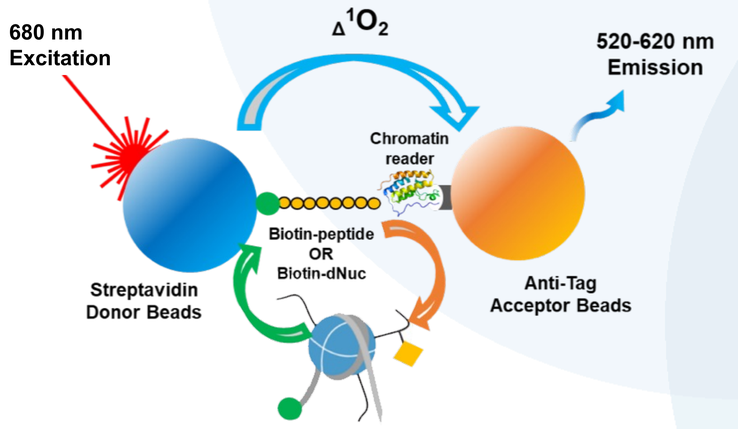
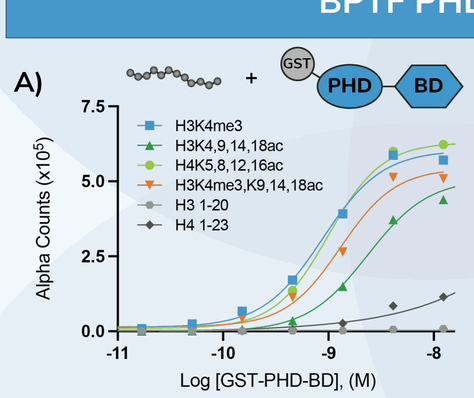


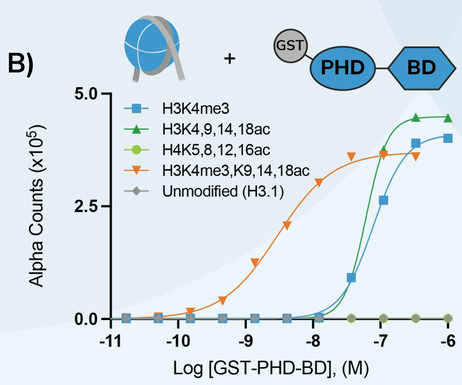
Figure 1. dCypher combines the benefits of peptides, nucleosomes and AlphaScreen® (Perkin Elmer) in one powerful assay. Above, a graphical depiction of dCypher: a no-wash liquid phase assay compatible with any biotinylated histone peptide or nucleosome. The approach has been optimized for use with GST-, 6HIS-, or FLAG-tagged query proteins.

BPTF PHD-BD Dual Domain Binds Cooperatively to Nucleosomes



C)

Peptide	EC_{50}^{rel}, nM
H3K4me3	0.9
H3K4,9,14,18ac	2.4
H4K5,8,12,16ac	1.0
H3K4me3,K9,14,18ac	1.3
H3 1-20	141
H4 1-23	29



D)

Nucleosome	EC_{50}^{rel}, nM
H3K4me3	78.0
H3K4,9,14,18ac	61.0
H4K5,8,12,16ac	ND
H3K4me3,K9,14,18ac	3.0
Unmodified (H3.1)	ND

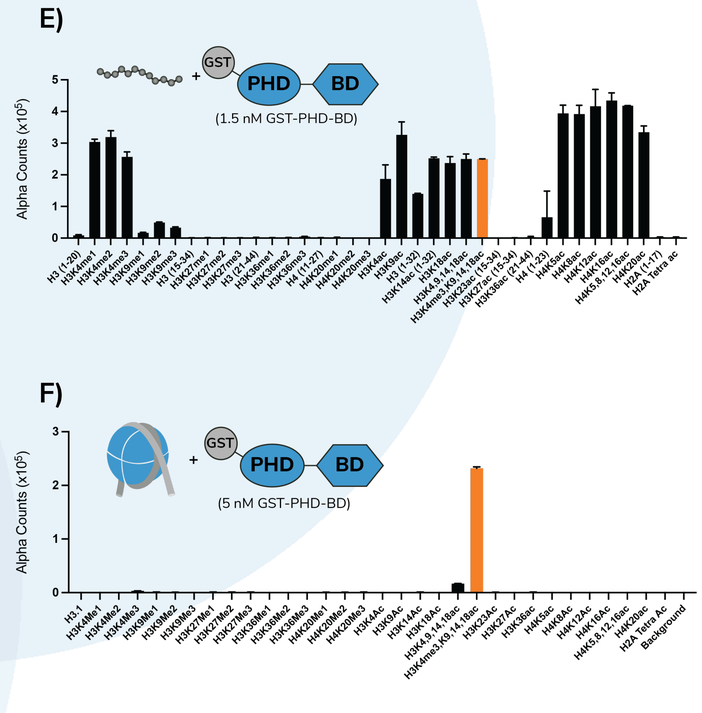
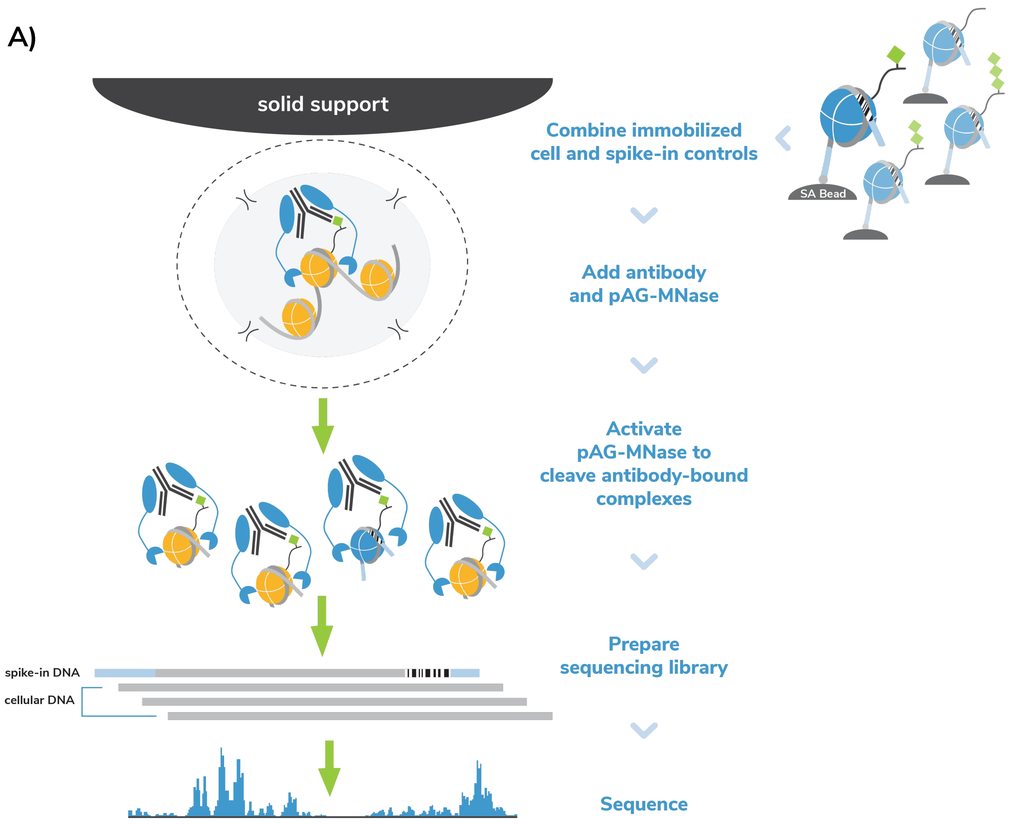


Figure 2. BPTF PHD-BD binds H3K4me3,K9,14,18ac cooperatively on nucleosomes. **A)** Titration of GST-BPTF PHD-BD against H3 and H4 histone peptides. Robust binding observed against methyl and acetyl peptides, consistent with expected domain function, though no cooperative binding was observed. **B)** Titration of GST-BPTF PHD-BD against H3 and H4 modified nucleosomes. Strong combinatorial engagement is observed on the H3K4me3,K9,14,18ac substrate (orange). **C,D)** Relative EC_{50} values from A & B determine optimal query protein concentration for E & F. **E)** Representative data from a 287-member dCypher discovery screen shows broad binding to H3K4methyl and H3/H4 acetyl peptides. **F)** Representative data from a 65-member nucleosome screen shows highly selective engagement with H3K4me3,K9,14,18ac.

CONCLUSIONS

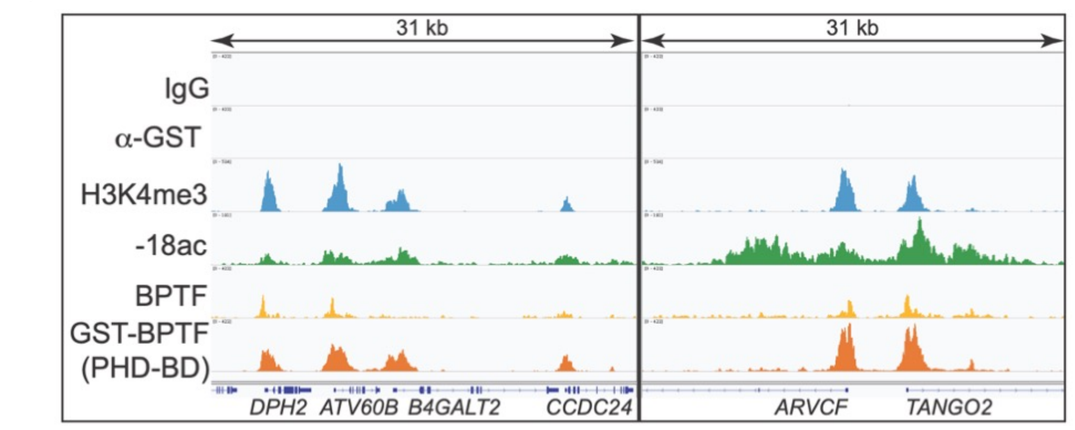
- ➢dCypher™ has major benefits compared to histone peptide arrays
- ➢BPTF PHD-BD cooperatively engages me & ac PTMs on nucleosomes <u>but not</u> peptides
- ➢Nucleosome context is critical to decipher the histone code
- ➢QUESTION: Do nucleosomes model *in vivo* specificity?

Reader-CUT&RUN Confirms PHD-BD Combinatorial Engagement *In Vivo*



C)

Spike-in Nuc Standards	Background Controls: IgG	Background Controls: α-GST	PTM Controls: H3K4me3	PTM Controls: -18ac	BPTF	GST-BPTF (PHD-BD)
WT	20%	19%	0%	1%	21%	2%
H3-TriTri	35%	31%	55%	67%	33%	82%
H3-tetraAc	26%	35%	1%	30%	18%	2%
H3K4me3	20%	15%	43%	2%	27%	13%
Nuc bandwidth	0.9%	0.7%	6.0%	1.0%	0.7%	1.8%



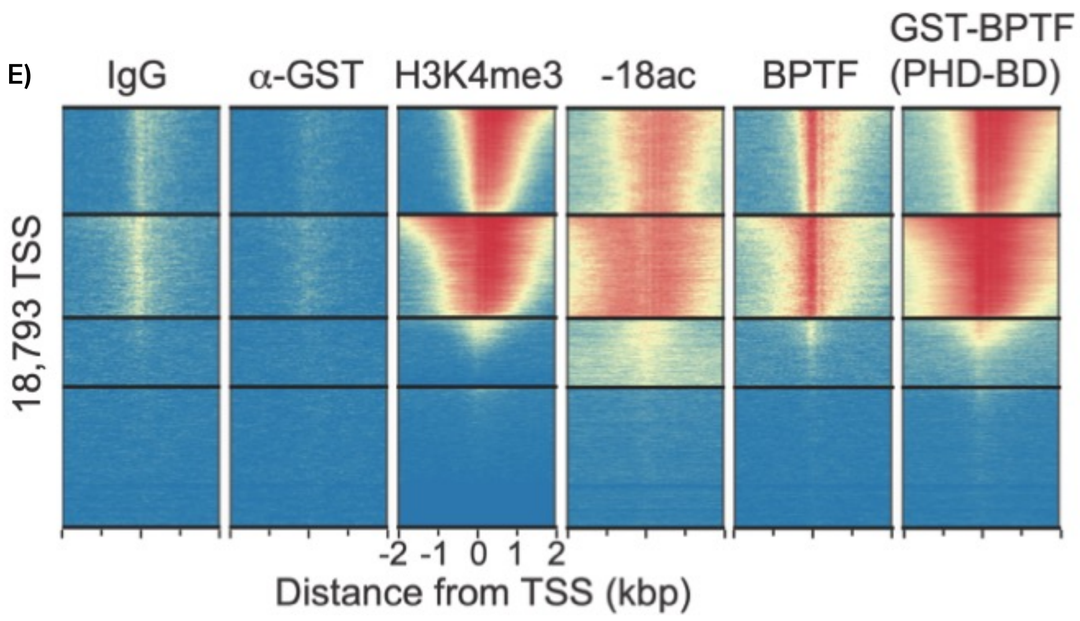


Figure 3. A) Graphical overview of the CUTANA® CUT&RUN protocol with SNAP-CUTANA™ spike-ins. **B)** Endogenous BPTF was probed using a BPTF antibody, while exogenous GST-PHD-BD was probed using anti-GST. **C)** Nucleosome spike-ins affirm dCypher GST-PHD-BD specificity with H3-TriTri spike-in enrichment (H3K4me3,K9,14,18ac). **D)** Sequenced DNA shows endogenous and exogenous BPTF map to locations containing both H3K4me3 and H3K18ac, supporting the observed nucleosome specificity in dCypher. The enhanced selectivity of endogenous BPTF suggests additional regulatory elements within the full length complex. **E)** Clustered heat map representing global signal mapped to transcription start sites (TSS) illustrates a genome-wide correlation of combinatorial engagement.

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