Combinatorial interactions by the BPTF tandem PHD-BD show nucleosome conformation dictates the histone code

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Background

Histone post-translational modifications (PTMs) play a critical role in chromatin regulation. It has been proposed that these PTMs form localized 'codes' that are read by specialized regions (reader domains) in chromatin associated proteins (CAPs) to regulate downstream function. Substantial effort has been made to define [CAP : histone PTM] specificities, and thus decipher the histone code and guide epigenetic therapies. However, this has largely been done using the reductive approach of isolated reader domains and histone peptides, which cannot account for any higher order factors. Here we show that the [BPTF PHD finger and bromodomain : histone PTM] interaction is dependent on nucleosome context. The tandem reader selectively associates with nucleosomal H3K4me3 and H3K14ac or H3K18ac, a combinatorial engagement that despite being in *cis* is not predicted by peptides. This *in vitro* specificity of the BPTF tandem reader for PTM-defined nucleosomes is recapitulated in a cellular context. We propose that regulatable histone tail accessibility and the associated impact on binding potential of reader domains necessitates we refine the 'histone code' concept and interrogate it at the nucleosome level.

dCypher® enables comprehensive reader analysis

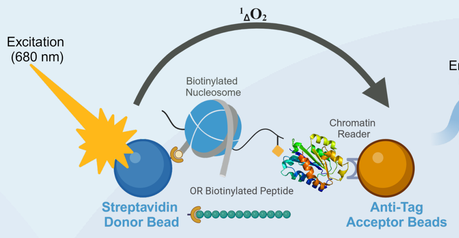


Figure 1. dCypher assay schematic.

- Robust signal-to-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

BPTF PHD-BD cooperatively binds to nucleosomes

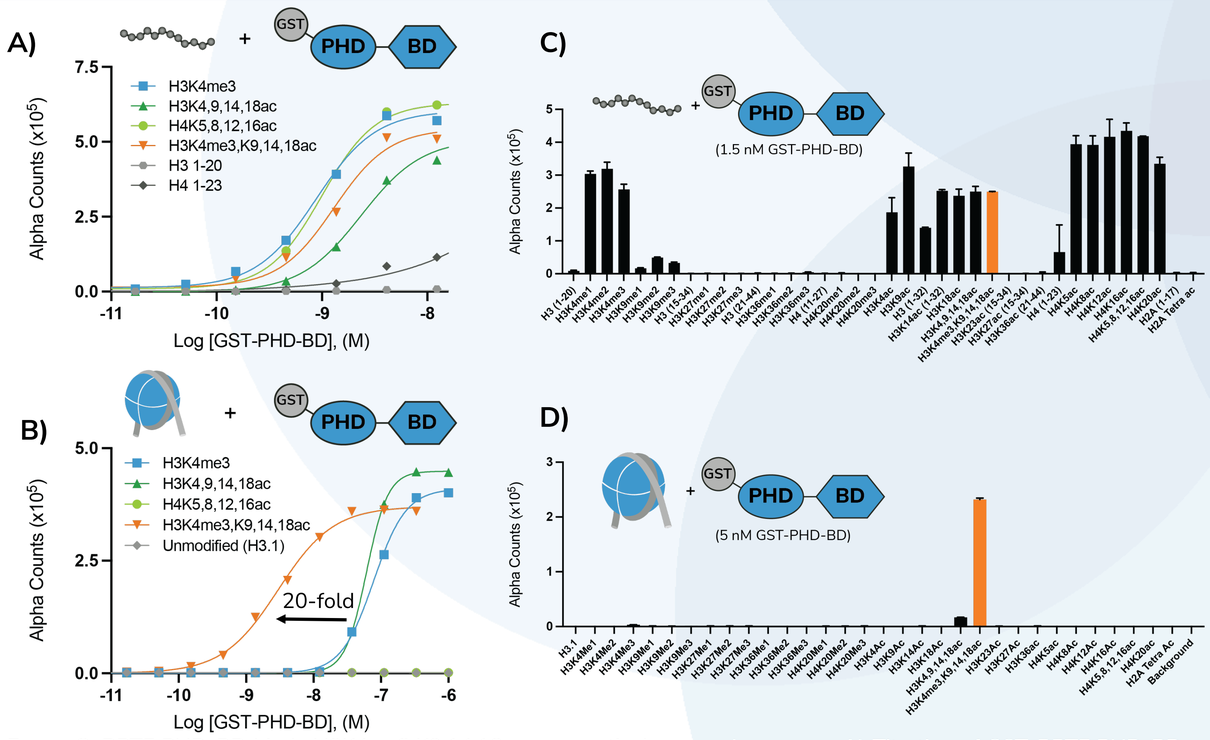


Figure 2. BPTF PHD-BD binds H3K4me3,K9,14,18ac cooperatively on nucleosomes. **A)** Titration of GST-BPTF PHD-BD against H3 and H4 histone peptides. Robust binding observed against methyl and acetyl peptides, consistent with expected domain function, though no cooperative binding was observed. **B)** 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)** Representative data from a 287-member dCypher discovery screen shows broad binding to H3K4methyl and H3/H4 acetyl peptides. **D)** Representative data from a 65-member nucleosome screen shows highly selective engagement with H3K4me3,K9,14,18ac.

Heterotypic dNucs™ show BPTF PHD-BD combinatorially binds in cis

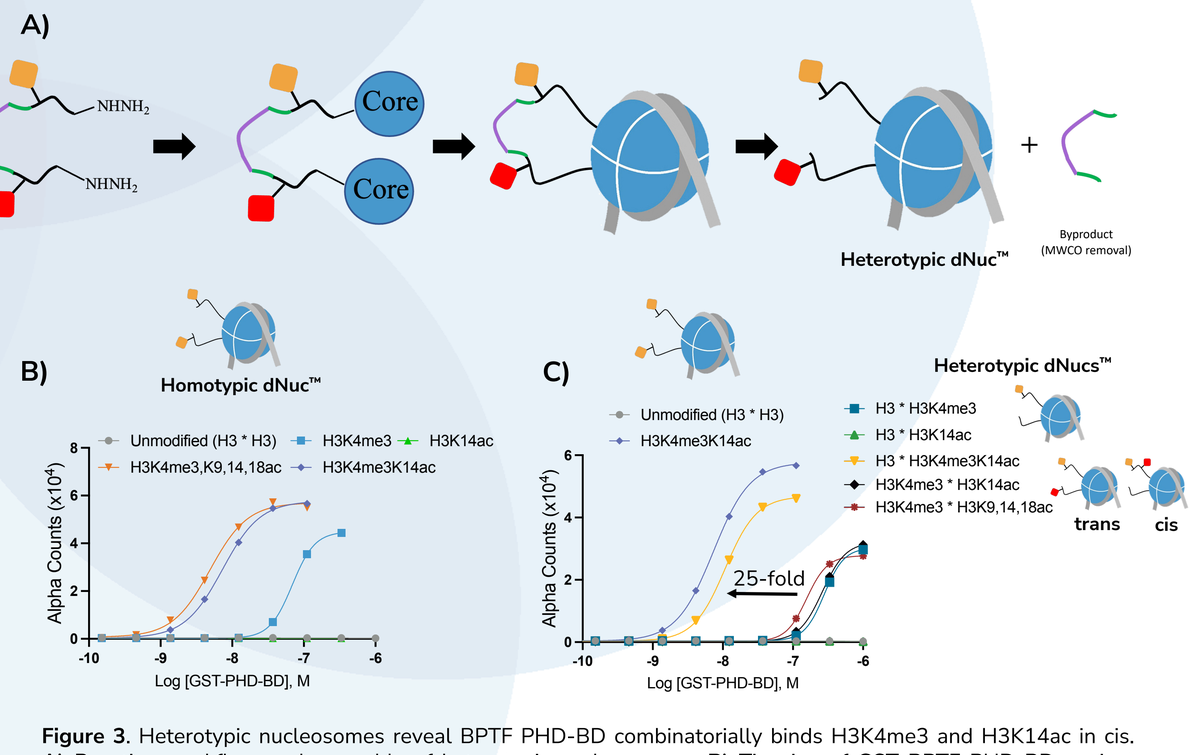
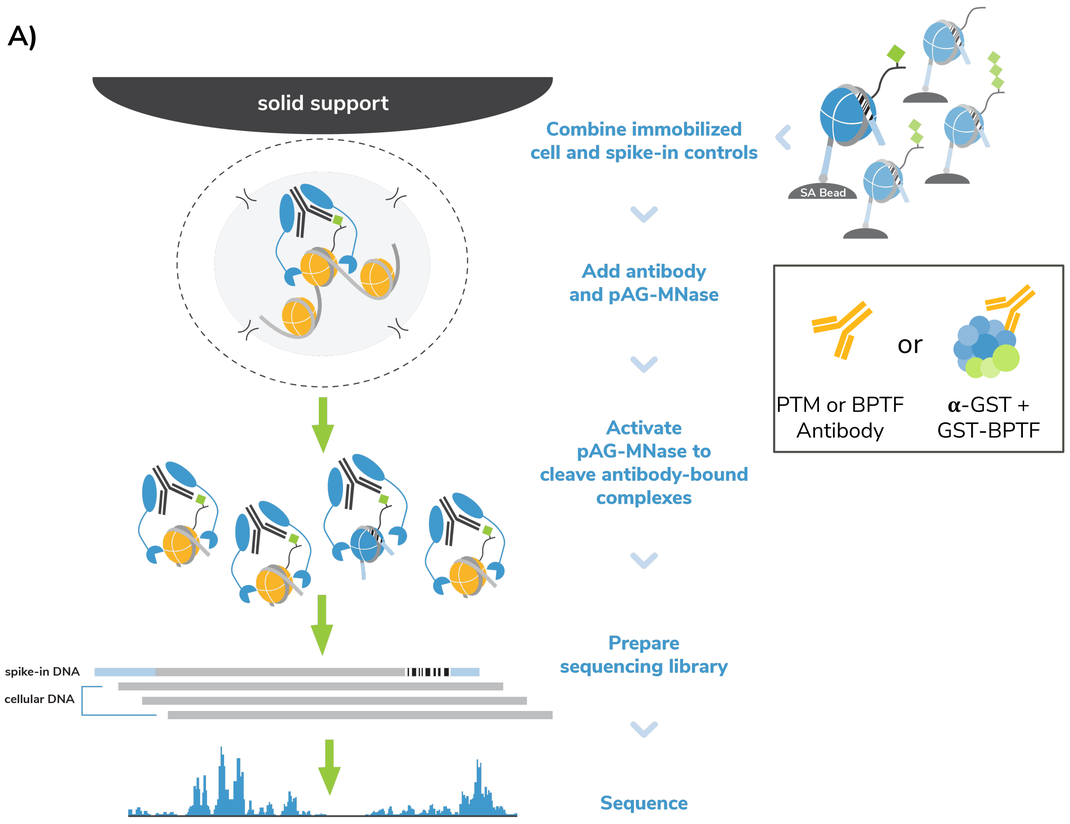


Figure 3. Heterotypic nucleosomes reveal BPTF PHD-BD combinatorially binds H3K4me3 and H3K14ac in cis. **A)** Reaction workflow and assembly of heterotypic nucleosomes. **B)** Titration of GST-BPTF PHD-BD against homotypic H3 modified nucleosomes. Strong combinatorial engagement was observed for H3K4me3,K9,14,18ac (orange) and H3K4me3,K14ac. **C)** Titration of GST-BPTF PHD-BD against heterotypic H3 modified nucleosomes. Strong combinatorial engagement is observed on [H3 * H3K4me3,K14ac] (cis configuration; light orange) but not [H3K4me3 * H3K14ac] (trans configuration; black) or [H3K4me3 * H3K9,14,18ac] (red).

Conclusions

- dCypher™ has major benefits compared to histone peptide arrays
- BPTF PHD-BD cooperatively engages PTMs in cis 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		PTM Controls			
	IgG	α-GST	H3K4me3	-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%

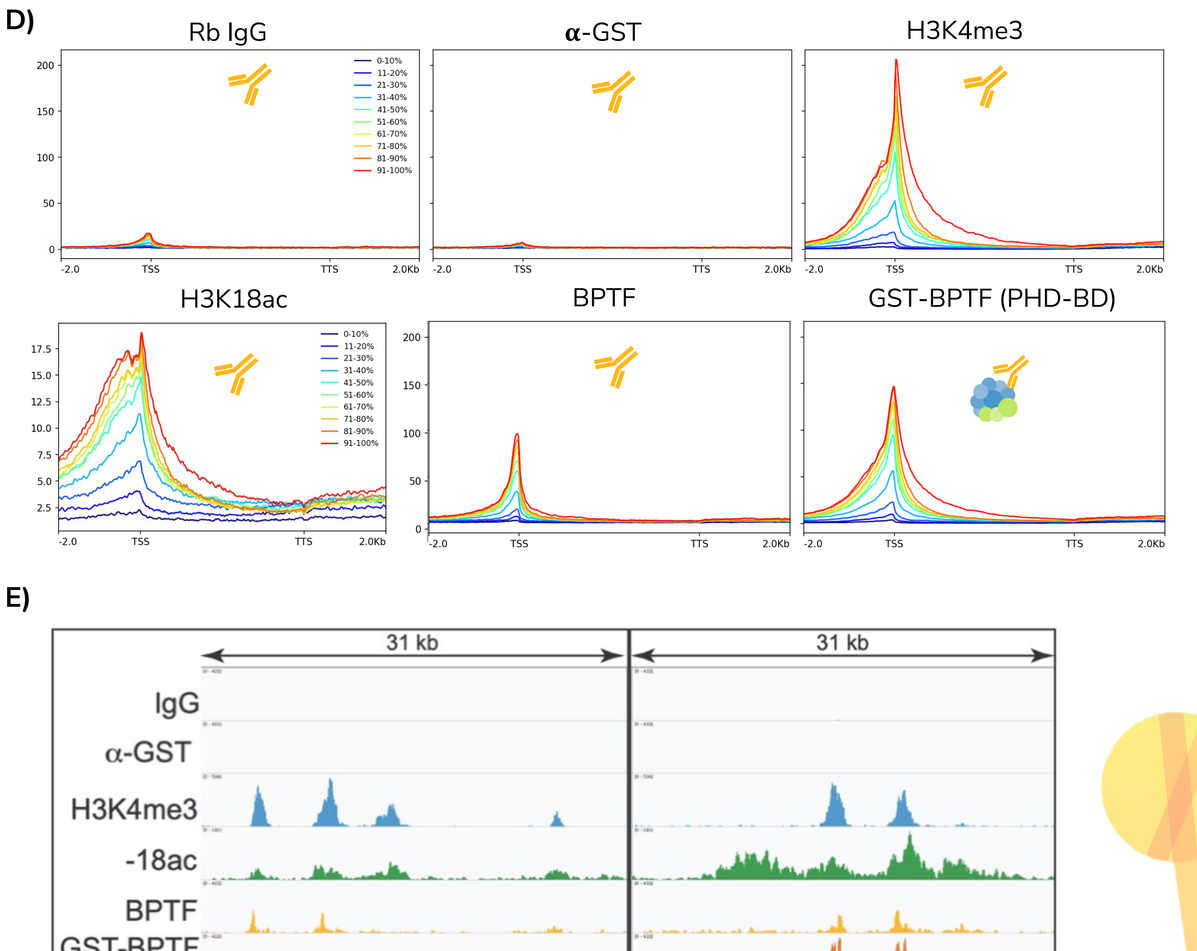


Figure 4. A) Graphical overview of the CUTANA® CUT&RUN protocol with SNAP-CUTANA™ spike-ins. **B)** Endogenous BPTF was probed using a BPTF antibody, while exogenous GST-PHD-BD was probed using anti-GST. **C)** Nucleosome spike-ins affirm dCypher GST-PHD-BD specificity with H3-TriTri spike-in enrichment (H3K4me3,K9,14,18ac). **D)** Signal mapped to gene bodies (TSS-TTS) ± 2 Kb according to RNAseq deciles illustrates a correlation with gene transcription. **E)** 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.

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