Development of a standards-driven genomic mapping platform to measure acute Pol II response dynamics

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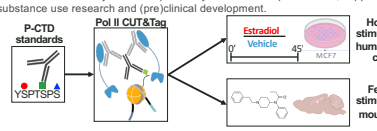
Abstract & Graphical Overview

Acute transcriptional responses are essential for normal development and homeostasis. However, current approaches to study transcription dynamics have significant limitations. To overcome these limitations, we have developed a CUT&Tag-based approach to directly and quantitatively profile chromatin-engaged RNA polymerase II (Pol II).

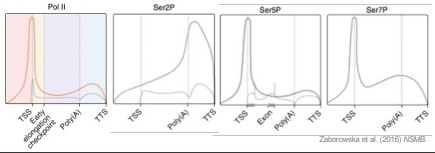
For assay development, we screened nearly 40 commercial Pol II antibodies for specificity / efficiency via our phospho-CTD-dNuc standards. Strikingly, for many antibodies, epitope recognition was weakened or completely masked when adjacent phospho-marks were present. In addition, most antibodies tested displayed a strong preference for the corresponding double modification versus its singly-modified counterpart.

The best performing reagents were used to benchmark Pol II CUT&Tag in MCF7 breast cancer cells stimulated by estradiol. Finally, optimized Pol II CUT&Tag was used to investigate transcriptional dynamics within an hour after opioid exposure. Cohorts of mice were treated with a fentanyl time-course; and brain tissue harvested, fixed and dissociated to isolate nuclei from striatal neurons. Subsequent Pol II CUT&Tag identified opioid-regulated genes and tracked their transcriptional dynamics over timed opioid exposure. Overall, Pol II CUT&Tag is a sensitive and scalable approach that will revolutionize the study of transcriptional dynamics in complex tissues, supporting substance use research and (pre)clinical development.



Distinguishing Pol II phospho-CTD dynamics critically depends upon antibody performance

Canonical CTD heptad: YSPTSPS
- 52x and 26x heptad repeats in human and yeast, respectively

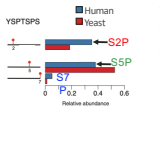


Zaborowska et al. (2016) *NSMB.*

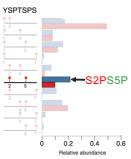
However, the specificity, efficiency, and masking effects of antibodies for Pol II CTD phospho-forms are not fully understood.

Phospho-CTD marks exist in all three forms: mono, dual, and tandem

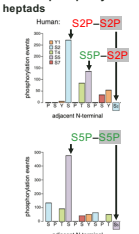
Mono-phosphorylated heptads



Dual-phosphorylated heptads



Tandem-phosphorylated heptads



Functional unit of RNA Pol II CTD lies within heptad-pairs
YSPTSPS–YSPTSPS

Stiller & Cook (2004) *Euk. Cell.*
Shah et al. (2019) *Biol. Lett.*
Appel et al. (2021) *Nat. Commun.*

Schüller et al. (2016) *Mol. Cell.*
Suh et al. (2016) *Mol. Cell.*

37 α-Pol II antibodies tested

15 Pol II | 22 Phospho-specific Pol II

6 RPB1 | 9 RPB3 | 3 S2/5P | 9 S2P | 7 S5P | 3 S7P

17 unique clones (+9 more soon)

17 monoclonal | 20 polyclonal

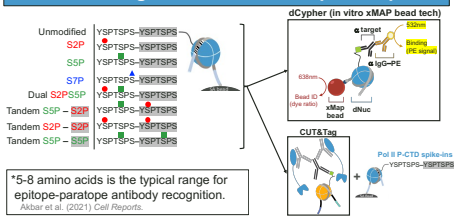
28 rabbit | 9 other

9 manufacturers

9x abcam | 6x ABclonal | 8x BETHYL | 3x invitrogen | 4x Cell Signaling TECHNOLOGY | 6x ACTIVE MOTIF

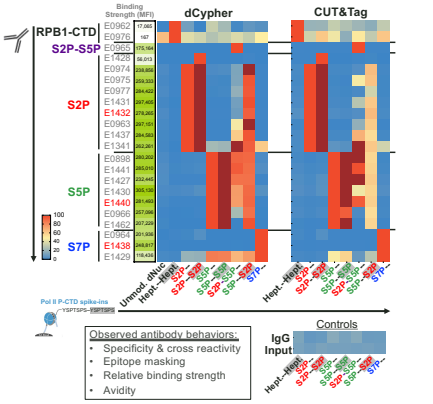
1x each Millipore, Rockland, Santa Cruz

Rational design of Pol II P-CTD spike-in panel



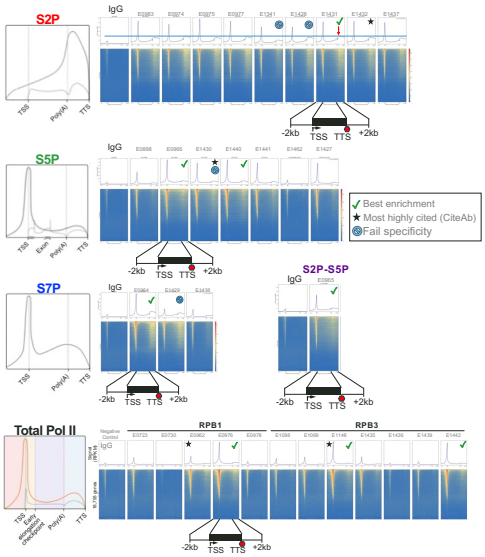
*5-8 amino acids is the typical range for epitope-paratope antibody recognition.
Akbar et al. (2021) *Cell Reports.*

Insights from Pol II antibody characterization: specificity, masking, and avidity revealed



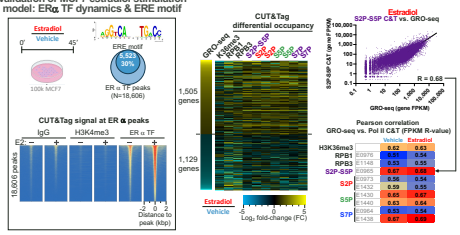
Observed antibody behaviors:
- Specificity & cross reactivity
- Epitope masking
- Relative binding strength
- Avidity

Pol II P-CTD enrichment patterns across gene bodies in light the conventional model

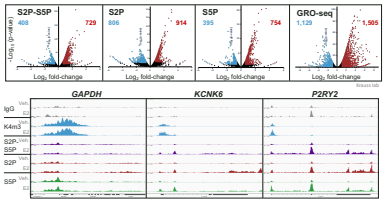


Pol II P-CTD CUT&Tag is a proxy for nascent transcription: using only 100k MCF7 cells

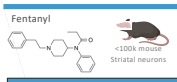
Validation of MCF7 estradiol stimulation model: ERα TF dynamics & ERE motif



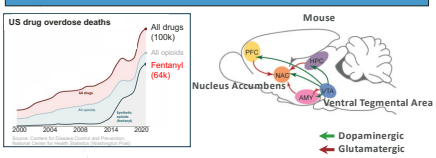
P-CTD dynamics in MCF7 estradiol model



Acute Pol II dynamics in fentanyl-stimulated neurons

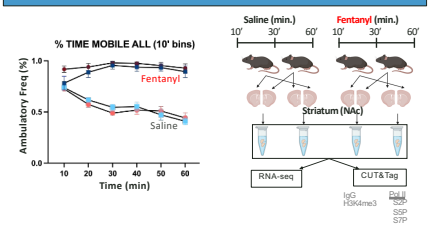


Dopamine reward circuit is central to the fentanyl overdose death crisis

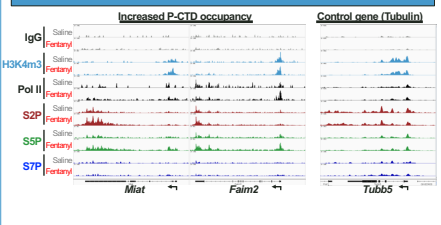


Walker and Nestler (2018) *Handb Clin Neurol.*

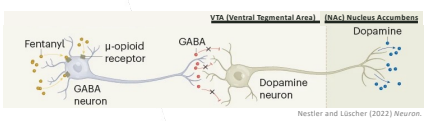
Mice injected with fentanyl become acutely hyperactive



Genome browser view of genes with increased P-CTD in response to fentanyl



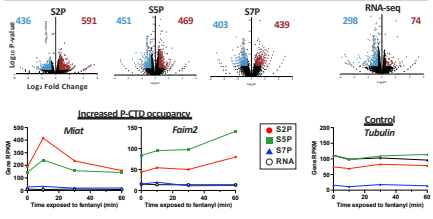
Fentanyl hijacks the dopamine reward circuit: signaling from VTA to NAc



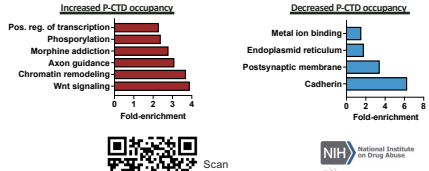
Nestler and Lüscher (2022) *Neuron.*

How does fentanyl impact transcriptional and epigenetic pathways in the NAcs?

Fentanyl stimulation: acute P-CTD dynamics detected in <100k cells, prior to RNA-seq



Pol II phosphorylation dynamics linked to morphine addiction circuit & neural plasticity



Scan for pdf of poster.

NIH National Institute on Drug Abuse
SBIR·STTR
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