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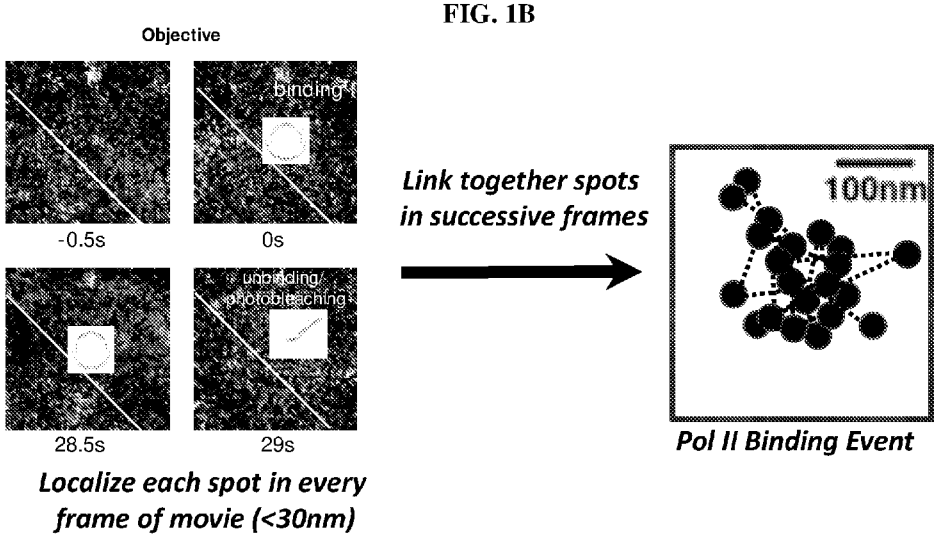
(54) Title: METHOD OF SCREENING FOR CHANGES IN ENZYMATIC ACTIVITY IN LIVE CELLS

FIG. 1B



(57) Abstract: The present disclosure describes methods of detecting, examining, and/or screening activity of a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin in a binding event that occurs in a live cell. The method includes performing live-cell single molecule tracking of the protein (e.g., an enzyme) in a binding event bound to the genome and/or chromatin of the cell at an image acquisition frame rate of about 300 to about 700 milliseconds/frame (e.g., about 400 to about 600 milliseconds/frame or about 500 milliseconds/frame) to generate 3 or more localization data points of binding events in the cell; and calculating the anisotropy formed between three successive localization data points. The methods of the present disclosure allow for a binding event, and activity during the same, of a protein to be examined.

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[Continued on next page]

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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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METHOD OF SCREENING FOR CHANGES IN ENZYMATIC ACTIVITY IN LIVE CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to, and the benefit of, U.S Provisional Application No. 63/497,235, filed 20 April 2023 and titled METHOD OF SCREENING FOR CHANGES IN ENZYMATIC ACTIVITY IN LIVE CELLS, which is incorporated by reference herein for all purposes.

FEDERAL RESEARCH STATEMENT

[0002] This invention was made with government support under 2R01GM126045 awarded by the National Institutes of Health/National Institute of General Medical Sciences. The government has certain rights in the invention.

FIELD OF THE DISCLOSURE

[0003] The present disclosure relates to methods of detecting, examining, and/or screening activity of an enzyme or protein, such as a modified (e.g., mutated) enzyme or protein, that binds and/or interacts with a genome and/or chromatin, as well as method of detecting, examining and/or screening the effect of an agent, such as a therapeutic agent, on an enzyme or protein, such as a modified (mutated) enzyme or protein.

BACKGROUND

[0004] Accurate and efficient screening of therapeutics requires rapid and sensitive assays that monitor target protein activity inside cells. Many therapeutic screens in cells are often based on an indirect readout of a target protein activity, which can result in both high rates of false negative hits and false positive hits.

[0005] Live cell single molecule imaging (LcSMI) can produce detailed kinetic binding profiles of a protein and/or enzyme associated with the genome or chromatin. Once bound to the genome and/or chromatin, the motion of proteins and/or enzymes is confined by positional oscillations of the genome and enzyme movement along the genome (Figures 1A and 1B). This motion is potentially affected by many factors including enzyme activity, deoxyribonucleic acid

(DNA) structure (e.g. single stranded versus double stranded and supercoiling) and locally associated factors (e.g. bound regulators and neighboring chromatin).

[0006] Prior work has used LcSMI at fast image acquisition rates (e.g. every about 10 milliseconds) to measure the anisotropy (e.g. biased directional movement, Figure 2A) of a nuclear diffusing factor 11-zinc finger protein (CTCF) as it engages its genomic target. Importantly, mutations in CTCF alters its engagement with its genomic target and corresponding changes in anisotropy. While fast LcSMI is quite powerful for examining engagement of factors with their genomic and/or chromatin targets, researchers are unable to define the activity of bound factors with this technique.

[0007] Polybromo-associated BRG1 or Pbrm-associated factor (PBAF) is a multi-subunit chromatin remodeling complex that both repositions and evicts nucleosomes via adenosine triphosphate (ATP) hydrolysis. Two key subunits within PBAF are polybromo (BAF180) and SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4 or BRG1). PBAF interacts with DNA packaging protein Histone H3 with an acetylated histone tail (H3K14ac). Currently, the arrangement of the BAF180 bromodomains (BD) 1-6 and the BRG1 BD are speculative.

Table 1. *In vitro* binding activity of BRG1 bromodomains for H3K14ac

Bromodomain of BRG1	**H3K14ac Binding**
BD2	+
BD3	-
BD2-BD3	-
BD3-BD4	-
BD4	+
BD4-BD5	++
BD5	-

Table 1 is reproduced from Slaughter MJ, *et al.* PBRM1 bromodomains variably influence nucleosome interactions and cellular function. J Biol Chem. 2018 Aug 31;293(35):13592-13603.

[0008] Some have developed assays measuring a protein's diffusion or movement using acquisition of 100 frames/sec or 10 msec/frame. See, e.g, Tjian, *et al. C&EN*, **2021**, 99(42):36–37. Tjian's assay measures a protein's diffusion under fast imaging conditions where it is typical for a fluorescent dye to last approximately 10 seconds since it requires high laser power illumination, thereby providing information related to the percentage of target protein that is bound and unbound to the genome. Such an assay is fast, which allows for an increased screening depth (10,000 - 30,000 compounds in a few days). However, the data resulting from the fast imaging is limited to whether or not a compound inhibits binding of a factor to the genome. The ~10 second assay window of Tjian is insufficient to obtain an anisotropy fingerprint related to a protein's physiological activity, which often occurs on timescales of tens of seconds to minutes. So while Tjian's assay is focused on speed and depth of screening a large number of compounds, the assay fails to pick up the anisotropic fingerprint related to a protein's physiological activity.

[0009] There accordingly remains a need in the art for methods capable of examining/defining the activity of genome and/or chromatin bound proteins and/or enzymes, such as examining/defining the activity with and/or without a therapeutic that is contacted/applied to said factors. The present disclosure describes a highly sensitive and rapid method for screening changes in the activity of a target protein and/or enzyme in live cells based upon the quantification of a protein's anisotropic movement when bound to its genomic/chromatin target(s).

SUMMARY

[0010] Presently described are methods for detecting, examining, and/or screening activity of an enzyme or protein, such as a modified (e.g., mutated) enzyme or protein, that binds or interacts with a genome and/or chromatin, as well as method of detecting, examining and/or screening the effect of an agent, such as a therapeutic agent, on an enzyme or protein, such as a modified (mutated) enzyme or protein. The inventors of the present disclosure surprisingly discovered a method with improved sensitivity for monitoring time dependent changes in anisotropy after a protein (e.g., an enzyme) binds the genome and/or chromatin in a live cell to examine the activity of the protein (e.g., enzyme) or a protein that interacts with the protein (e.g., enzyme), which can be used to examine, detect, and/or screen agents for their

activity for the enzyme or protein being examined. The methods described herein are also less labor intensive and time consuming then heretofore methods of examining protein and/or enzyme activity, including method of screening agents in live cells.

[0011] Thus, in an aspect, the present disclosure provides a method of detecting, examining, and/or screening activity of a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin in a binding event that occurs in a live cell, the method comprising: (a) performing live-cell single molecule tracking of the protein (e.g., enzyme) in a binding event bound to the genome and/or chromatin of the cell at an image acquisition frame rate of about 300 to about 700 milliseconds/frame (e.g., about 400 to about 600 milliseconds/frame or about 500 milliseconds/frame) to generate 3 or more localization data points of binding events in the cell; and (b) calculating the anisotropy formed between three successive localization data points (e.g., calculating the anisotropy formed between three successive localization data points during the trajectory).

[0012] Another aspect of the present disclosure provides a method of detecting, examining, and/or screening in a live cell the effect of a modification on a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin, the method comprising: preparing a cell with a wild-type protein; preparing a cell with a modified protein (e.g., a mutated version of the protein or a post-translationally modified version of the protein); performing steps (a) and (b) as described herein for (i) the cell with wild-type protein and (ii) the cell with the modified protein.

[0013] A further aspect of the present disclosure provides a method of detecting, examining, and/or screening a subject for a mutated version of a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin in a live cell, the method comprising: obtaining a cell with a version of the protein to be examined; performing steps (a) and (b) as described herein for the obtained cell.

[0014] An additional aspect of the present disclosure provides a method of detecting, examining, and/or screening in a live cell the effect of an agent on a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin, the method comprising: performing steps (a) and (b) as described herein on (i) a cell with a protein (e.g., an enzyme), (ii) a cell with a wild-type protein (e.g., a wild-type enzyme), (iii) a cell with a version of the protein to be examined (e.g., mutated version of the protein or enzyme or a post-translationally modified

version of the protein or enzyme), (iv) the version of the protein (e.g., enzyme) to be examined, or (v) a combination thereof; performing steps (a) and (b) on (i) a cell with a protein (e.g., an enzyme), (ii) a cell with a wild-type protein (e.g., a wild-type enzyme), (iii) a cell with the modified protein (e.g., mutated version of the protein or enzyme or a post-translationally modified version of the protein or enzyme), (iv) the version of the protein (e.g., enzyme) to be examined, or (v) a combination thereof, that has been contacted with an agent (e.g., a therapeutic agent, such as a small molecule, a peptide, a protein, siRNA, miRNA, shRNA, antisense RNA, RNAzyme, or DNAzyme, etc.).

[0015] Yet a further aspect of the present disclosure provides a method of detecting, examining, and/or screening activity of a protein that binds or interacts with a genome and/or chromatin in a binding event that occurs in a live cell, the method comprising: (1) performing live-cell single molecule tracking of an imaged protein (e.g., an imaged enzyme) in a binding event that interacts with the protein and is bound to the genome and/or chromatin of the cell at an image acquisition frame rate of about 300 to about 700 milliseconds/frame (e.g., about 400 to about 600 milliseconds/frame or about 500 milliseconds/frame) to generate 3 or more localization data points of binding events in the cells; and (2) calculating the anisotropy formed between three successive localization data points (e.g., calculating the anisotropy formed between three successive localization data points during the trajectory), wherein the anisotropy of the imaged protein is indicative of the interaction of the protein with the genome and/or chromatin.

[0016] Yet another aspect of the present disclosure provides a method of detecting, examining, and/or screening in a live cell the effect of a modification on a protein that binds or interacts with a genome and/or chromatin, the method comprising: preparing a cell with a wild-type protein; preparing a cell with a modified protein (e.g., a mutated version of the protein or a post translationally modified version of the protein); performing steps (1) and (2) as described herein for (i) the cell with wild-type protein and (ii) the cell with the modified protein.

[0017] Yet a further aspect of the present disclosure provides a method of detecting, examining, and/or screening a subject for a mutated version of a protein that binds or interacts with a genome and/or chromatin in a live cell, the method comprising: obtaining a cell with a version of the protein to be examined; performing steps (1) and (2) as described herein for the obtained cell.

[0018] Yet an additional aspect of the present disclosure provides a method of detecting, examining, and/or screening in a live cell the effect of an agent on a protein that binds or interacts with a genome and/or chromatin, the method comprising: performing steps (1) and (2) as described herein on (i) a cell with a protein, (ii) a cell with a wild-type protein, (iii) a cell with a modified protein (e.g., mutated version of the protein or a post-translationally modified version of the protein), (iv) the version of the protein to be examined, or (v) a combination thereof; performing steps (1) and (2) as described herein on (i) a cell with a protein, (ii) a cell with a wild-type protein, (iii) a cell with a modified protein (e.g., mutated version of the protein or a post translationally modified version of the protein), (iv) the version of the protein to be examined, or (v) a combination thereof, that has been contacted with an agent (e.g., a therapeutic agent, such as a small molecule, a peptide, a protein, siRNA, miRNA, shRNA, antisense RNA, RNAzyme, or DNAzyme, etc.).

[0019] The preceding general areas of utility are given by way of example only and are not intended to be limiting on the scope of the present disclosure and appended claims. Additional objects and advantages associated with the compositions, methods, and processes of the present disclosure will be appreciated by one of ordinary skill in the art in light of the instant claims, description, and examples. For example, the various aspects and embodiments of the present disclosure can be utilized in numerous combinations, all of which are expressly contemplated by the present disclosure. These additional advantages objects and embodiments are expressly included within the scope of the present disclosure. The publications and other materials used herein to illuminate the background of the invention, and in particular cases, to provide additional details respecting the practice, are incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The accompanying drawings, which are incorporated into and form a part of the specification, illustrate several embodiments of the present disclosure and, together with the description, serve to explain the principles of the disclosure. The drawings are only for the purpose of illustrating an embodiment of the disclosure and are not to be construed as limiting the disclosure. Further objects, features and advantages of the disclosure will become apparent from the following detailed description taken in conjunction with the accompanying figures showing illustrative embodiments of the disclosure.

[0021] **Figures 1A and 1B.** Overview of Live Cell Single Molecule Imaging (LcSMI). (FIG. 1A) Motion Blur Highly inclined and Laminated optical sheet (HiLo) microscopy of proteins tagged with a HaloTag or SNAP-tag®, fluorescently labeled with a bright organic fluorophore that is capable of being tracked over extended periods of times (about 60 to about 200 seconds) at an image acquisition rate of about 0.5 seconds/frame before photobleaching. (FIG. 1B) Ribonucleic acid (RNA) Polymerase II containing SNAP-RPB1 molecules that rapidly diffuse in the nucleoplasm are blurred, while chromatin bound RNA Polymerase II appears as single bright spots (highlighted by circles). Disappearance of a spot (white arrow) is due to unbinding or dissociation of an RNA Polymerase II from the chromatin. A diagonal white line is an added spatial reference that is positioned at the same location in each frame. Spots in close proximity in successive frames (less than or about 500 nm) are linked together to generate a binding trajectory (right).

[0022] **Figures 2A, 2B, and 2C.** Overview of Anisotropy Measurements to Monitor for Changes in the Activity of Genomic Bound RNA Polymerase II. (FIG. 2A) The angle formed between three temporally successive localizations in a binding trajectory is calculated. Angles are accumulated across binding trajectories for many molecules in many cells and used to generate an angular anisotropy histogram plot, as shown in FIG. 2B. (FIG. 2B) Angular anisotropy histogram plot showing a slight leftward skew of molecules moving forward and back displaying anisotropic movement. (FIG. 2C) Schematic showing that an elongating RNA Polymerase II (left) bound to the genome has the potential to generate less anisotropic movement compared to a paused stationary RNA Polymerase II (right) whose movement is dominated by the oscillatory motion of the genome. In addition, elongating RNA Polymerase bound to a semi-rigid constrained genome in close proximity to barrier proteins, such as nucleosomes, has the potential to generate more anisotropic movement compared to RNA Polymerase II bound to a more slack genome further away from a barrier protein whose movement is dominated by the oscillatory motion of the intervening genome between RNA Polymerase II and the barrier protein.

[0023] **Figure 3.** Alterations in the Time Dependent Anisotropy Profile of deoxyribonucleic acid (DNA) Polymerase δ Upon Mutational Inactivation. Anisotropy measurements were taken of SNAP-tagged wild-type DNA Polymerase δ (upper line) or catalytically dead DNA Polymerase δ (lower line) binding events in human LOX cells. Binding

events were filtered using 13 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis). Mutational inactivation leads to a general elevation of anisotropy values over time.

[0024] **Figure 4.** Alterations in the Time Dependent Anisotropy Profile of RNA Polymerase II Upon Mutational Activation. Anisotropy measurements were taken of wild-type RNA Polymerase II/SNAP-RPB1 (upper line) or fast E1126G mutant RNA Polymerase II/SNAP-RPB1 (lower line) in U2OS cells. Binding events were filtered using 11 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis). Acceleration of RNA Polymerase II elongation rates via mutation results in an overall decrease in the anisotropy profile over time.

[0025] **Figure 5.** Small Molecule Inhibition of Multiple Regulators of Pol II Results in Distinct Time Dependent Anisotropy Profiles. Anisotropy measurements were taken of wild-type RNA Polymerase II/SNAP-RPB1 after treatment with dimethyl sulfoxide/control, Flavopiridol/CDK9 (cyclin-dependent kinase 9) inhibitor THZ1/CDK7 (cyclin-dependent kinase 7) inhibitor, and Triptolide/xeroderma pigmentosum type B (XPB) inhibitor in U2OS cells. Binding events were filtered using 11 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis).

[0026] **Figures 6A, 6B, 6C, 6D, and 6E.** Small Molecule Inhibition or Mutation of Multiple domains of BRG1-Associated Factor 180 (BAF180) subunit and the SMARCA4 or BRG1 subunit of the ATP-dependent Polybromo-associated BAF (PBAF) Results in Distinct Time Dependent Anisotropy Profiles. FIG. 6A, Domain schematic of BAF180 and SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4 or BRG1). Individual BAF180 bromodomains (BDs) differentially interact with DNA packaging protein Histone H3 with an acetylated histone tail (H3K14ac)-containing nucleosomes *in vitro*. The arrangement of the six bromodomains of BAF180 (BD1-6) and BRG1 Bromodomain are speculative. FIG. 6B illustrates an anisotropy analysis schematic. The genome fluctuates like a harpsichord. Therefore, the activity of PBAF can be assessed by measuring the propensity of Halo-BAF180 to recoil back over three successive frames. Stalled PBAF will have a strong directional bias (higher f180/0), thereby reflecting a complex with less activity. Active PBAF will have less directional bias and have a lower f180/0 value. FIG. 6C, Anisotropy measurements taken of wild-type PBAF, Halo-BAF180 WT control and PFI3

treated along with mutant PBAF containing 6 bromodomains deleted Halo-BAF180 ΔBD in U2OS cells. Binding events were filtered using 11 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis). FIG. 6D, Anisotropy of BAF180 containing distinct point mutations that inactivate acetyl-lysine binding. Mutation of BD5 (N739F) causes PBAF to stall and be less active, whereas mutation of BD3 (N463F) causes PBAF to be more active. These results agree with experiments performed *in vitro*, where BD5 was found to enhance PBAF's association with H3K14ac-containing nucleosomes, while BD3 represses binding. FIG. 6E,Anisotropy of BAF180 containing a BD5 mutation with and without BRG1 BD inhibition. As shown in Figure 6E, the N739F mutation causes BAF180 to become less active. However, additional inhibition of the BRG1 bromodomain results in substantial decreases in anisotropy. These changes reflect a complex communication between the BAF180 and BRG1 BDs.

[0027] **Figure 7.** Wild-Type or Cancer Mutant tumor protein P53 (p53) Differentially Alters the Time Dependent Anisotropy Profile of Pol II. Anisotropy measurements were taken of wild-type RNA polymerase II/SNAP-RPB1 after induction of wild-type p53 or cancer mutant p53 R273H in U2OS cells. A control lacking induction of p53 was also examined. Binding events were filtered using 11 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis).

[0028] **Figure 8.** Wild-Type or Cancer Mutant p53 Differentially Alters the Time Dependent Anisotropy Profile of EZH2. Anisotropy measurements were taken of Halo-tagged EZH2 after induction of wild-type p53 or cancer mutant p53 R248W in U2OS cells. Binding events were filtered using 11 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis).

[0029] **Figures 9A, 9B, 9C, and 9D**. Overview of generation of temporal activity profiles. (9A) Temporal activity heat map showing Pol II binding events and angles at each time point after Pol II arrival on the genome. Each horizontal line is a single Pol II binding event. Black color denotes molecules at moved with an angle of between 150-180° at a given time point. White color denotes molecules at moved with an angle of between 31-149° at a given time point. Hatch pattern denotes molecules at moved with an angle of between 0.1-30° at a given time point. (9B) Sample schematic showing assignment of angles and colors or pattern for time points of individual molecules. (9C) Schematic showing calculation of f180/0 values at

a series of single time points across a number of individual binding events. (9D) Temporal Activity profile of Pol II showing anisotropy fluctuations as Pol II loads onto the genome (1 to about 3.7 seconds) followed by a regular pattern of oscillatory fluctuations over time that likely represents Pol II molecules that engage the template DNA, but are unable to productively move into the gene body (about 5 to about 55 seconds).

[0030] **Figures 10A, 10B, 10C, and 10D**. Alterations in the Temporal Activity Profiles after small molecule inhibition of Pol II. (10A) Schematic showing the kinetic steps involved in Pol II promoter escape and elongation into the gene body. Note that the times shown for CDK7 mediated release of MED and Pol II pause release are derived from FRAP studies on Pol II. (10B) Temporal activity profile of Pol II bound between 30 and 40 seconds in the absence (closed circles) and presence (open triangle) of Triptolide, which inhibits transcription factor IIH (TFIIH)/xeroderma pigmentosum type B (XPB) mediated single stranded DNA (ssDNA) bubble formation. Note the relative lack of temporal fluctuations in anisotropy for Pol II after it loads onto the genome (>3.7 seconds). (10C) Temporal activity profile of Pol II bound for long lived events (closed circle, >60 seconds and open triangle, 60-70 seconds). Note that early temporal fluctuations of anisotropy become suppressed as Pol II moves from a predicted poised state to a more elongating form that likely enters into the gene body. (10D) Temporal activity profile of Pol II bound for long lived events (>60) in the absence (close circle) and presence (open triangle) of the CDK9 inhibitor NVP-1. Note that there are relatively minimal changes in temporal fluctuations between the control and NVP-1 treated samples before 50 seconds. However, there are significantly stronger and more frequent fluctuations at about 85 to about 90 seconds after Pol II arrives on the genome in the presence of NVP-1. This suggests that Pol II promoter escape and pause release may take a significant amount of time to occur after Pol II recruitment to a promoter.

[0031] **Figure 11A and 11B**. Alterations in the temporal activity profiles of poly [adenosine diphosphate-ribose] polymerase 1 (PARP1) under dysregulation of cellular oxidized nicotinamide adenine dinucleotide (NAD+) levels. (11A) Temporal activity profile (left) and standard deviation of mean temporal activity profile (right) of a short lived (30 to 40 seconds) interaction of PARP1 with the genome. Note that the f180/0 value for the first time point (1 second) significantly deviates between the PARP in high NAD+ versus low NAD+ cells. Such a rapid difference early in binding suggests that the genomic/chromatin scaffold may be

fundamentally different when cells are metabolically dysregulated. Such shifts caused by differences in the genomic/chromatin scaffolds can be normalized by determining the standard deviation of f180/0 at each time point relative to the mean of f180/0 for all timepoints. Note that temporal activity is the greatest during initial loading of PARP1 onto the genome and during disassembly of PARP1 from the genome with relatively little NAD+ dependent fluctuations in the interim. (11B) Temporal activity profile (left) and standard deviation of mean temporal activity profile (right) of a long lived (60 to 70 seconds) interaction of PARP1 with the genome. Note the there are many significant NAD+ dependent temporal fluctuations in anisotropy of PARP1 when bound to the genome for long periods of time. This may suggest many rounds of enzymatic activity of PARP1 during such long live genomic binding events (60 to 70 seconds) relative to a short lived binding event (<40 seconds).

DETAILED DESCRIPTION

[0032] The present disclosure will now be described more fully hereinafter, but not all embodiments of the disclosure are shown. While the disclosure has been described with reference to exemplified embodiments, it will be understood by those skilled in the art that various changes can be made and equivalents can be substituted for elements thereof without departing from the scope of the disclosure. In addition, many modifications can be made to adapt a particular structure or material to the teachings of the disclosure without departing from the essential scope thereof.

[0033] Where a range of values is provided, it is understood that each intervening value between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges can independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the present disclosure.

[0034] The following terms are used to describe the present invention. In instances where a term is not specifically defined herein, that term is given an art-recognized meaning by those of ordinary skill applying that term in context to its use in describing the present invention.

[0035] The articles "a" and "an" as used herein and in the appended claims are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article unless the context clearly indicates otherwise. By way of example, "an element" means one element or more than one element.

[0036] The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements can optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0037] As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e., "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of."

[0038] As used herein in the specification and in the claims, "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from anyone or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those

elements specifically identified. Thus, as a nonlimiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[0039] In the claims, as well as in the specification above, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," "composed of," and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the 10 United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

[0040] As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from anyone or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements can optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a nonlimiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc. It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

[0041] Presently described are methods for detecting, examining, and/or screening activity of an enzyme or protein, such as a modified (e.g., mutated) enzyme or protein, that binds or interacts with a genome and/or chromatin, as well as method of detecting, examining and/or screening the effect of an agent, such as a therapeutic agent, on an enzyme or protein, such as a modified (mutated) enzyme or protein. The inventors of the present disclosure surprisingly discovered a method with improved sensitivity for monitoring time dependent changes in anisotropy after a protein (e.g., an enzyme) binds a genome and/or chromatin in a live cell to examine the activity of the protein (e.g., enzyme) or a protein that interacts with the imaged protein (e.g., imaged enzyme), which can be used to examine, detect, and/or screen agents for their activity for the enzyme or protein being examined. The methods described herein are also less labor intensive and time consuming than heretofore methods of examining protein and/or enzyme activity, including method of screening agents in live cells.

[0042] In particular, using live cell single molecule imaging (LcSMI) at slow image acquisition rates (e.g., every about 500 milliseconds), time dependent changes in the anisotropy profile of protein molecules bound for different times to their genomic targets. The method of the present disclosure, also referred to herein as genome bound anisotropy (GEANIS), is a significantly improved way to screen for therapeutics and examiner cell lines and patient samples. The method of the present disclosure collects individual binding events over a rolling window, such as a rolling 11-second window, and determines the fold anisotropy (f180/0) for all localizations within those elected binding events. A further refinement and improvements to the method of the present disclosure to map out temporal changes in the activity of proteins at select time points after they have bound their genomic/chromatin targets. This method is also referred to herein as temporal activity genome bound anisotropy (taGEANIS). The information provided by the method can be utilized to further define the mechanism of action of various therapeutics during screening.

[0043] An advantage of the inventions of the present disclosure is that changes in a protein's anisotropic movement can be tracked as it is performing its physiological activity on a timescale of seconds to minutes. This in turn gives a much broader window (5 to 150 seconds) to determine if and/or how a therapeutic may act on a target protein.

[0044] As mentioned in the background, Tjian's method only examine proteins/enzymes that bind to chromatin/genome since both utilize an expected change in protein diffusion upon

binding a chromatin/genome scaffold as a readout. This is similar to the current inventive methods in that both methods can identify compounds that change the percentage of target proteins bound to chromatin/genome. The inventions of the present disclosure, however, possess the major advantage of also being able to identify compounds or mutations that do not inhibit genomic binding but do affect a target protein's activity once bound to the genome. The Tjian method cannot identify compounds or mutations that do not affect the percentage of genomic binding of target protein but can alter protein activity once bound to the genome.

[0045] Inventions of the present disclosure, therefore, provide more detailed temporal information on how (e.g. mechanism of action) a compound or a mutation impacts a target protein's physiological activity once bound to the genome. Other assays, such as the Tjian method, only provide a limited amount of information on if a compound or mutation disrupts binding of the target protein to the genome.

[0046] Thus, in an aspect, the present disclosure provides a method of detecting, examining, and/or screening activity of a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin in a binding event that occurs in a live cell, the method comprising: (a) performing live-cell single molecule tracking of the protein (e.g., the enzyme) in a binding event bound to the genome and/or chromatin of the cell at an image acquisition frame rate of about 300 to about 700 milliseconds/frame (e.g., about 400 to about 600 milliseconds/frame or about 500 milliseconds/frame) to generate 3 or more localization data points of binding events in the cell; and (b) calculating the anisotropy formed between three successive localization data points (e.g., calculating the anisotropy formed between three successive localization data points during the trajectory).

[0047] In any aspect or embodiment described herein, the present invention provides an assay that can measure movement, such as anisotropic movement. For example, in any aspect or embodiment described herein, the anisotropic movement examined is a fold anisotropy, f180/0, the ratio of the percentage of proteins that move forward or back compared with enzymes that move only forward in successive time frames (e.g. anisotropic movement is indicated by an F180/0 ratio greater than 1).

[0048] In any aspect or embodiment described herein, performing live-cell single molecule tracking at about 25°C to about 37°C. For example, in any aspect or embodiment described herein, performing live-cell single molecule tracking at about 25°C to about 37°C,

about 25°C to about 36°C, about 25°C to about 35°C, about 25°C to about 34°C, about 25°C to about 33°C, about 25°C to about 32°C, about 25°C to about 31°C, about 25°C to about 30°C, about 25°C to about 29°C, about 25°C to about 28°C, about 25°C to about 27°C, about 26°C to about 37°C, about 26°C to about 36°C, about 26°C to about 35°C, about 26°C to about 34°C, about 26°C to about 33°C, about 26°C to about 32°C, about 26°C to about 31°C, about 26°C to about 30°C, about 26°C to about 29°C, about 26°C to about 28°C, about 27°C to about 37°C, about 27°C to about 36°C, about 27°C to about 35°C, about 27°C to about 34°C, about 27°C to about 33°C, about 27°C to about 32°C, about 27°C to about 31°C, about 27°C to about 30°C, about 27°C to about 29°C, about 28°C to about 37°C, about 28°C to about 36°C, about 28°C to about 35°C, about 28°C to about 34°C, about 28°C to about 33°C, about 28°C to about 32°C, about 28°C to about 31°C, about 28°C to about 30°C, about 29°C to about 37°C, about 29°C to about 36°C, about 29°C to about 35°C, about 29°C to about 34°C, about 29°C to about 33°C, about 29°C to about 32°C, about 29°C to about 31°C, about 30°C to about 37°C, about 30°C to about 36°C, about 30°C to about 35°C, about 30°C to about 34°C, about 30°C to about 33°C, about 30°C to about 32°C, about 31°C to about 37°C, about 31°C to about 36°C, about 31°C to about 35°C, about 31°C to about 34°C, about 31°C to about 33°C, about 32°C to about 37°C, about 32°C to about 36°C, about 32°C to about 35°C, about 32°C to about 34°C, about 33°C to about 37°C, about 33°C to about 36°C, about 33°C to about 35°C, about 34°C to about 37°C, about 34°C to about 36°C, or about 35°C to about 37°C.

[0049] In any aspect or embodiment described herein, performing live-cell single molecule tracking for up to about 10 or about 20 minutes. For example, in any aspect or embodiment described herein, performing live-cell single molecule tracking for about 1 to about 20, about 1 to about 18, about 1 to about 16, about 1 to about 14, about 1 to about 12, about 1 to about 10, about 1 to about 8, about 1 to about 6, about 1 to about 4 minutes, about 2 to about 20, about 2 to about 18, about 2 to about 16, about 2 to about 14, about 2 to about 12, about 2 to about 10, about 2 to about 8, about 2 to about 6, about 2 to about 4 minutes, about 4 to about 20, about 4 to about 18, about 4 to about 16, about 4 to about 14, about 4 to about 12, about 4 to about 10, about 4 to about 8, about 4 to about 6, about 6 to about 20, about 6 to about 18, about 6 to about 16, about 6 to about 14, about 6 to about 12, about 6 to about 10, about 6 to about 8, about 8 to about 20, about 8 to about 18, about 8 to about 16, about 8 to about 14, about 8 to

about 12, about 10 to about 12, about 10 to about 20, about 10 to about 18, about 10 to about 16, about 10 to about 14, about 10 to about 12, about 12 to about 20, about 12 to about 18, about 12 to about 16, about 12 to about 14, about 14 to about 20, about 14 to about 18, about 14 to about 16, about 16 to about 20, about 16 to about 18, about 18 to about 20.

[0050] For example, in any aspect or embodiment described herein, performing live-cell single molecule tracking for about 1 to about 10, about 1 to about 9, about 1 to about 8, about 1 to about 7, about 1 to about 6, about 1 to about 5, about 1 to about 4, about 1 to about 3, about 1 to about 2, about 2 to about 10, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4, about 2 to about 3, about 3 to about 10, about 3 to about 9, about 3 to about 8, about 3 to about 7, about 3 to about 6, about 3 to about 5, about 3 to about 4, about 4 to about 10, about 4 to about 9, about 4 to about 8, about 4 to about 7, about 4 to about 6, about 4 to about 5, about 5 to about 10, about 5 to about 9, about 5 to about 8, about 5 to about 7, about 5 to about 6, about 6 to about 10, about 6 to about 9, about 6 to about 8, about 6 to about 7, about 7 to about 10, about 7 to about 9, about 7 to about 8, about 8 to about 10, about 8 to about 9, or about 9 to about 10 minutes).

[0051] In any aspect or embodiment described herein, performing live-cell single molecule tracking includes illuminating the cell for about 200 to about 700 millisecond (e.g., about 200 to about 700, about 200 to about 600, about 200 to about 500, about 200 to about 400, about 200 to about 300, about 300 to about 700, about 300 to about 600, about 300 to about 500, about 300 to about 400, about 400 to about 700, about 400 to about 600, about 400 to about 500, about 500 to about 700, about 500 to about 600, or about 600 to about 700) every about 1 to about 4 seconds (e.g., about 1 to about 4, about 1 to about 3.5, about 1 to about 3, about 1 to about 2.5, about 1 to about 2, about 1 to about 1.5, about 1.5 to about 4, about 1.5 to about 3.5, about 1.5 to about 3, about 1.5 to about 2.5, about 1.5 to about 2, about 2 to about 4, about 2 to about 3.5, about 2 to about 3, about 2 to about 2.5, about 2.5 to about 4, about 2.5 to about 3.5, about 2.5 to about 3, about 3 to about 4, about 3 to about 3.5, or about 3.5 to about 4 seconds).

[0052] In any aspect or embodiment described herein, performing live-cell single molecule tracking comprises: performing two-dimensional imaging (e.g., fluorescent microscopy); performing live-cell single molecule tracking or two-dimensional imaging includes illuminating a protein for about 60 to about 200 seconds (e.g., about 60 to about 200,

about 60 to about 180, about 60 to about 160, about 60 to about 140, about 60 to about 120, about 60 to about 100, about 80 to about 200, about 80 to about 180, about 80 to about 160, about 80 to about 140, about 80 to about 120, about 80 to about 100, about 100 to about 200, about 100 to about 180, about 100 to about 160, about 100 to about 140, about 100 to about 120, about 120 to about 200, about 120 to about 180, about 120 to about 160, about 120 to about 140, about 140 to about 200, about 140 to about 180, about 140 to about 160, about 160 to about 200, about 160 to about 180, or about 180 to about 200); performing live-cell single molecule tracking or two-dimensional imaging on about 8 to about 17 cells (e.g., about 10 to about 15, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 cells) in a single field of view; the protein is a protein comprising a mutation (e.g., a mutated protein or a mutated enzyme); the protein comprises a label (e.g., a fluorescent marker or label), an affinity tag (e.g., an affinity tag, His tag (metal ions, such as Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}), biotin (streptavidin), Strep II Tag (Streptavidin or Strep-Tactin), biotin (Streptavidin), calmodulin-binding peptide (CBP) tag (calmodulin), chitin-binding domain (CBD) tag (chitin), maltose-binding protein (MBP) (amylose), glutathione S-transferase (GST) tag (glutathione), S-tag (S-protein of RNase A), FLAG tag (DYKDDDDK) (anti-FLAG monoclonal antibod, such as M1, M2, and M5, or a derivative thereof), HaloTag (a reactive chloroalkane linker), SNAP-tag®(e.g., guanine or chloropyrimidine with a benzyl linker to the label)), or a combination thereof; or a combination thereof.

[0053] In any aspect or embodiment described herein, the protein comprises an affinity tag and a fluorescent label is covalently linked to a ligand of the affinity tag; the method further comprises contacting the cell with a fluorescent label covalently linked to a ligand of an affinity tag, wherein the protein comprises the affinity tag; or a combination thereof.

[0054] In any aspect or embodiment described herein, two-dimensional imaging is performed with a camera (e.g., electron-multiplying charged-coupled device (EMCCD) camera, scientific complementary metal-oxide-semiconductor (sCMOS) camera, or a combination thereof) that has a resolution of about 60 to about 100 nm/pixel (e.g., about 65 to about 95 nm/pixel).

[0055] In any aspect or embodiment described herein, performing live-cell single molecule tracking further comprises preparing the trajectory comprises: (1) residence time of the protein (i.e., the time of binding to the genome or chromatin), (2) average two-dimensional

(XY) position of the protein, (3) the two-dimensional (XY) positions of the protein over successive frames, or (4) a combination thereof; fitting (e.g., 2D Gaussian fitting) individual labels in a frame at a resolution of less than about 35 nanometers (nm) (e.g., less than about 30, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 5 to about 35, about 5 to about 30, about 5 to about 25, about 5 to about 20, about 5 to about 15, about 10 to about 35, about 10 to about 30, about 10 to about 25, about 10 to about 20, about 15 to about 35, about 15 to about 30, about 15 to about 25, about 20 to about 35, about 20 to about 30, or about 25 to about 35 nm); linking localizations in successive frames that are within about 500 nm to form an individual binding trajectory or trajectories; removing genomic and/or chromatic localizations (e.g., the average two-dimensional (XY) position) of the protein that are within about 2 or less microns (e.g., about 0 to about 2 microns) over a period of at least about 8 seconds (e.g., about 8 seconds to about 14 seconds or about 11 seconds); calculating the angle between at least three (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) successive localizations from a residence time window or successive residence time windows (e.g., a window of about 8 to about 20 seconds or about 8 to about 14 seconds, such as 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 seconds) of the individual binding trajectory or trajectories, wherein each successive residence time window is shifted by about 1, about 2, about 3, about 4, or about 5 seconds (e.g., with a 1 second shift, a first window that is 2 to about 12 seconds of the individual binding trajectory or trajectories, a second window that is about 3 to about 13 seconds of the individual binding trajectory or trajectories, a third window that is about 4 to about 14 seconds of the individual binding trajectory or trajectories, a fourth window that is about 5 to about 15 seconds of the individual binding trajectory or trajectories, a fifth window that is about 6 to about 16 seconds of the individual binding trajectory or trajectories, etc.); or a combination thereof.

[0056] In any aspect or embodiment described herein, calculating the anisotropy formed between three successive localization data points (e.g., calculating the anisotropy formed between three successive localization data points during the trajectory) comprises: determining if the genome and/or chromatin bound protein displays random (e.g., isotropic) or a biased (anisotropic) movement by examining angular measurements (e.g., by graphing the angular measurements, such as on a polar histogram plot) from at least 5 cells (e.g., 5, 6, 7, 8, 9, 10, or more cells) in a given residence window; graphing a plurality of cells (e.g., at least 5 cells, such

as 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 cells) in each resident time window on a polar histogram plot; determining the percentage of proteins that move two-dimensionally (e.g., back and forth or oscillatory) with those that only move forward in one dimension in successive frames; quantifying the anisotropic movement as the fold anisotropy (e.g., as the f180/0 ratio, as the percentage of proteins that move forward or back compared with proteins that only move forward in successive frames); preparing a time dependent anisotropy profile (e.g., by plotting the fold anisotropy for each residence time window verse the centroid of the residence time window); or a combination thereof.

[0057] In any aspect or embodiment described herein, calculating the anisotropy formed between three successive localization data points comprises: generating a temporal angular signature for a genome and/or chromatin binding event (e.g., one or more, or each, genome and/or chromatin binding event) from one or more cells (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 cells); the temporal angular signature of a genome and/or chromatin binding event comprises, consists essentially of, or consists of, a series of angles after the arrival of the protein onto the genome and/or chromatin (e.g., the first angle of the series of angles is the arrival of the protein onto the genome and/or chromatin), wherein each angle is calculated from 3 localization data points (e.g., 3 successive localization data points); the temporal angular signature comprises, consists essentially of, or consists of, a residence time window (e.g., a window of about 8 to about 20 seconds or about 8 to about 14 seconds, such as 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 seconds) of the individual binding trajectory or trajectories after the arrival of the protein onto the genome and/or chromatin (e.g., the first angle of the series of angles is the arrival of the protein onto the genome and/or chromatin); quantifying the anisotropic movement of the temporal angular signature for the genome and/or chromatin binding event (e.g., a series of angles after the arrival of the protein onto the genome and/or chromatin) as the fold anisotropy (e.g., as the f180/0 ratio, as the percentage of proteins that move forward or back compared with proteins that only move forward in successive frames); generating a temporal activity profile for a genome and/or chromatin binding event (e.g., one or more, or each, genome and/or chromatin binding event) by plotting the fold anisotropy (e.g., the f180/0 ratio) of the temporal angular signature for the genome and/or chromatin binding event as a function of time (e.g., a function of time after the arrival of the protein onto the genome and/or chromatic); comparing temporal activity profiles from different

binding events (e.g., different time periods, residence time windows, successive residence time windows, etc.); or a combination thereof.

[0058] In any aspect or embodiment described herein, the method further comprises: expressing the protein in the cell (e.g., incubating the cell that expresses the protein for about 5 hours to about 30 hours, such as under acceptable growth conditions (e.g., about 36 to 38°C at 5%CO_2)); mutating the protein; labeling or tagging the protein (e.g., an affinity tag (such as HaloTag or SNAP-tag®), a fluorescent label, etc.); contacting the cell with a fluorescent label that is covalently linked to a ligand of an affinity tag, wherein the protein comprises the affinity tag; or a combination thereof.

[0059] In any aspect or embodiment described herein, contacting comprises: contacting the cell with the fluorescent label covalently linked to the ligand of the affinity tag is for about 15 to about 30 minutes (e.g., about 15 to about 30, about 15 to about 25, about 15 to 20, about 20 to about 30, about 20 to about 25, of about 25 to about 30 minutes) before performing live-cell single molecule tracking of the protein; contacting the cell with the fluorescent label covalently linked to the ligand of the affinity tag is performed with about 0.5 to about 5.0 nM (e.g., about 0.5 to about 5.0, about 0.5 to about 4.5, about 0.5 to about 4.0, about 0.5 to about 3.5, about 0.5 to about 3.0, about 0.5 to about 2.5, about 0.5 to about 2.0, about 0.5 to about 1.5, about 1.0 to about 5.0, about 1.0 to about 4.5, about 1.0 to about 4.0, about 1.0 to about 3.5, about 1.0 to about 3.0, about 1.0 to about 2.5, about 1.0 to about 2.0, about 1.5 to about 5.0, about 1.5 to about 4.5, about 1.5 to about 4.0, about 1.5 to about 3.5, about 1.5 to about 3.0, about 1.5 to about 2.5, about 2.0 to about 5.0, about 2.0 to about 4.5, about 2.0 to about 4.0, about 2.0 to about 3.5, about 2.0 to about 3.0, about 2.5 to about 5.0, about 2.5 to about 4.5, about 2.5 to about 4.0, about 2.5 to about 3.5, about 3.0 to about 5.0, about 3.0 to about 4.5, about 3.0 to about 4.0, about 3.5 to about 5.0, about 3.5 to about 4.5, or about 4.0 to about 5.0) of the fluorescent label; or a combination thereof.

[0060] Another aspect of the present disclosure provides a method of detecting, examining, and/or screening in a live cell the effect of a modification on a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin, the method comprising: preparing a cell with a wild-type protein; preparing a cell with a modified protein (e.g., a mutated version of the protein or enzyme or a post-translationally modified version of the

protein or enzyme); performing steps (a)and (b) as described herein for (i) the cell with wild-type protein and (ii) the cell with the modified protein.

[0061] In any aspect or embodiment described herein, the method further comprising comparing the anisotropy formed between three successive localization data points (e.g., comparing the anisotropy formed between three successive localization data points during the trajectory) of the wild-type protein and the mutated protein.

[0062] In any aspect or embodiment described herein, the modified protein is a mutated version of the wild-type protein.

[0063] In any aspect or embodiment described herein, preparing the cell with the modified protein comprises mutating the wild-type protein (mutated protein or enzyme) in a cell.

[0064] In any aspect or embodiment described herein, preparing the cell with the modified protein: obtaining cells from a subject (such as, a hematopoietic stem cell(s) or a cancer cell(s)); growing the obtained cell or cells or replacing the protein of a cell with the protein from the obtained cell or cells; and performing steps (a) and (b) on the obtained cell(s) or the cell(s) with the protein from the obtained cell(s).

[0065] A further aspect of the present disclosure provides a method of detecting, examining, and/or screening a subject for a mutated version of a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin in a live cell, the method comprising: obtaining a cell with a version of the enzyme to be examined; performing steps (a) and (b) as described herein for the obtained cell.

[0066] In any aspect or embodiment described herein, the method further comprising comparing the anisotropy formed between three successive localization data points (e,g., comparing the anisotropy formed between three successive localization data points during the trajectory) of the cell with the version of the protein to be examined and a cell comprising a wild-type protein (e.g., a matched cell comprising the wild-type protein or enzyme).

[0067] An additional aspect of the present disclosure provides a method of detecting, examining, and/or screening in a live cell the effect of an agent on an enzyme that binds or interacts with a genome and/or chromatin, the method comprising: performing steps (a) through (c) as described herein on (i) a cell with a protein (e.g., an enzyme), (ii) a cell with a wild-type protein (e.g., a wild-type enzyme), (iii) a cell with a version of the protein to be examined (e.g.,

mutated version of the protein or enzyme or a post-translationally modified version of the protein or enzyme), (iv) the version of the protein (e.g., enzyme) to be examined, or (v) a combination thereof; performing steps (a) and (b) on (i) a cell with a protein (e.g., an enzyme), (ii) a cell with a wild-type protein (e.g., a wild-type enzyme), (iii) a cell with the modified protein (e.g., mutated version of the protein or enzyme or a post-translationally modified version of the protein or enzyme), (iv) the version of the protein (e.g., enzyme) to be examined, or (v) a combination thereof, that has been contacted with an agent (e.g., a therapeutic agent, such as a small molecule, a peptide, a protein, siRNA, miRNA, shRNA, antisense RNA, RNAzyme, or DNAzyme, etc.).

[0068] In any aspect or embodiment described herein, the method further comprises: contacting the cell with the agent; calculating the anisotropy or angle formed between three temporally successive localizations during a trajectory of a genomic binding event of the protein, wild-type protein, the version of the protein to be examined, and/or mutated protein in the cell contacted with the agent and/or a chromatin binding event of the protein, wild-type protein, and/or mutated protein in the cell contacted with the agent; and comparing the anisotropy or angle formed between three temporally successive localizations of (i) the protein, the wild-type protein, the version of the protein to be examined, and/or the modified protein (e.g., mutated version of protein or enzyme or a post-translationally modified version of the protein or enzyme) in the cell not contacted with the agent, and (ii) the protein, the wild-type protein, the version of the protein to be examined, and/or the modified protein (e.g., mutated version of protein or enzyme or a post-translationally modified version of the protein or enzyme) in the cell contacted with the agent.

[0069] In any aspect or embodiment described herein, the agent is an inhibitor of a chromatin remodeling complex (e.g., BRG1 or hbrm-associated factor (BAF) or polybromo-associated BRG1 or Pbrm-associated factor (PBAF)), an RNA polymerase (e.g., RNA polymerase I, II, and/or III), a DNA polymerase (e.g., DNA polymerase β (beta), λ (lamda), σ (sigma), μ (mu), α (alpha), δ (delta), ε (epsilon), η (eta), ι (iota), κ (kappa), Rev1, ζ (zeta), γ (gamma), θ (theta), and/or ν (nu)), a telomerase, or a reverse transcriptase.

[0070] In any aspect or embodiment described herein, the protein, the wild-type protein, the version of the protein to be examined, and/or the modified protein is an enzyme.

[0071] Yet a further aspect of the present disclosure provides a method of detecting, examining, and/or screening activity of a protein that binds or interacts with a genome and/or chromatin in a binding event that occurs in a live cell, the method comprising: (1) performing live-cell single molecule tracking of an imaged protein (e.g., the an imaged enzyme) in a binding event that interacts with the protein and is bound to the genome and/or chromatin of the cell at an image acquisition frame rate of about 300 to about 700 milliseconds/frame (e.g., about 400 to about 600 milliseconds/frame or about 500 milliseconds/frame) to generate 3 or more localization data points of binding events in the cells; and (2) calculating the anisotropy formed between three successive localization data points (e.g., calculating the anisotropy formed between three successive localization data points during the trajectory), wherein the anisotropy of the imaged protein is indicative of the interaction of the protein with the genome and/or chromatin.

[0072] In any aspect or embodiment described herein, performing live-cell single molecule tracking at about 25°C to about 37°C. For example, in any aspect or embodiment described herein, performing live-cell single molecule tracking at about 25°C to about 37°C, about 25°C to about 36°C, about 25°C to about 35°C, about 25°C to about 34°C, about 25°C to about 33°C, about 25°C to about 32°C, about 25°C to about 31°C, about 25°C to about 30°C, about 25°C to about 29°C, about 25°C to about 28°C, about 25°C to about 27°C, about 26°C to about 37°C, about 26°C to about 36°C, about 26°C to about 35°C, about 26°C to about 34°C, about 26°C to about 33°C, about 26°C to about 32°C, about 26°C to about 31°C, about 26°C to about 30°C, about 26°C to about 29°C, about 26°C to about 28°C, about 27°C to about 37°C, about 27°C to about 36°C, about 27°C to about 35°C, about 27°C to about 34°C, about 27°C to about 33°C, about 27°C to about 32°C, about 27°C to about 31°C, about 27°C to about 30°C, about 27°C to about 29°C, about 28°C to about 37°C, about 28°C to about 36°C, about 28°C to about 35°C, about 28°C to about 34°C, about 28°C to about 33°C, about 28°C to about 32°C, about 28°C to about 31°C, about 28°C to about 30°C, about 29°C to about 37°C, about 29°C to about 36°C, about 29°C to about 35°C, about 29°C to about 34°C, about 29°C to about 33°C, about 29°C to about 32°C, about 29°C to about 31°C, about 30°C to about 37°C, about 30°C to about 36°C, about 30°C to about 35°C, about 30°C to about 34°C, about 30°C to about 33°C, about 30°C to about 32°C, about 31°C to about 37°C, about 31°C to about 36°C, about 31°C to

about 35°C, about 31°C to about 34°C, about 31°C to about 33°C, about 32°C to about 37°C, about 32°C to about 36°C, about 32°C to about 35°C, about 32°C to about 34°C, about 33°C to about 37°C, about 33°C to about 36°C, about 33°C to about 35°C, about 34°C to about 37°C, about 34°C to about 36°C, or about 35°C to about 37°C.

[0073] In any aspect or embodiment described herein, performing live-cell single molecule tracking for up to about 10 or about 20 minutes. For example, in any aspect or embodiment described herein, performing live-cell single molecule tracking for about 1 to about 20, about 1 to about 18, about 1 to about 16, about 1 to about 14, about 1 to about 12, about 1 to about 10, about 1 to about 8, about 1 to about 6, about 1 to about 4 minutes, about 2 to about 20, about 2 to about 18, about 2 to about 16, about 2 to about 14, about 2 to about 12, about 2 to about 10, about 2 to about 8, about 2 to about 6, about 2 to about 4 minutes, about 4 to about 20, about 4 to about 18, about 4 to about 16, about 4 to about 14, about 4 to about 12, about 4 to about 10, about 4 to about 8, about 4 to about 6, about 6 to about 20, about 6 to about 18, about 6 to about 16, about 6 to about 14, about 6 to about 12, about 6 to about 10, about 6 to about 8, about 8 to about 20, about 8 to about 18, about 8 to about 16, about 8 to about 14, about 8 to about 12, about 10 to about 12, about 10 to about 20, about 10 to about 18, about 10 to about 16, about 10 to about 14, about 10 to about 12, about 12 to about 20, about 12 to about 18, about 12 to about 16, about 12 to about 14, about 14 to about 20, about 14 to about 18, about 14 to about 16, about 16 to about 20, about 16 to about 18, about 18 to about 20.

[0074] For example, in any aspect or embodiment described herein, performing live-cell single molecule tracking for about 1 to about 10, about 1 to about 9, about 1 to about 8, about 1 to about 7, about 1 to about 6, about 1 to about 5, about 1 to about 4, about 1 to about 3, about 1 to about 2, about 2 to about 10, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4, about 2 to about 3, about 3 to about 10, about 3 to about 9, about 3 to about 8, about 3 to about 7, about 3 to about 6, about 3 to about 5, about 3 to about 4, about 4 to about 10, about 4 to about 9, about 4 to about 8, about 4 to about 7, about 4 to about 6, about 4 to about 5, about 5 to about 10, about 5 to about 9, about 5 to about 8, about 5 to about 7, about 5 to about 6, about 6 to about 10, about 6 to about 9, about 6 to about 8, about 6 to about 7, about 7 to about 10, about 7 to about 9, about 7 to about 8, about 8 to about 10, about 8 to about 9, or about 9 to about 10 minutes).

[0075] In any aspect or embodiment described herein, performing live-cell single molecule tracking includes illuminating the cell for about 200 to about 700 millisecond (e.g., about 200 to about 700, about 200 to about 600, about 200 to about 500, about 200 to about 400, about 200 to about 300, about 300 to about 700, about 300 to about 600, about 300 to about 500, about 300 to about 400, about 400 to about 700, about 400 to about 600, about 400 to about 500, about 500 to about 700, about 500 to about 600, or about 600 to about 700) every about 1 to about 4 seconds (e.g., about 1 to about 4, about 1 to about 3.5, about 1 to about 3, about 1 to about 2.5, about 1 to about 2, about 1 to about 1.5, about 1.5 to about 4, about 1.5 to about 3.5, about 1.5 to about 3, about 1.5 to about 2.5, about 1.5 to about 2, about 2 to about 4, about 2 to about 3.5, about 2 to about 3, about 2 to about 2.5, about 2.5 to about 4, about 2.5 to about 3.5, about 2.5 to about 3, about 3 to about 4, about 3 to about 3.5, or about 3.5 to about 4 seconds).

[0076] In any aspect or embodiment described herein, performing live-cell single molecule tracking comprises: performing two-dimensional imaging (e.g., fluorescent microscopy); performing live-cell single molecule tracking or two-dimensional imaging includes illuminating a protein for about 60 to about 200 seconds (e.g., about 60 to about 200, about 60 to about 180, about 60 to about 160, about 60 to about 140, about 60 to about 120, about 60 to about 100, about 80 to about 200, about 80 to about 180, about 80 to about 160, about 80 to about 140, about 80 to about 120, about 80 to about 100, about 100 to about 200, about 100 to about 180, about 100 to about 160, about 100 to about 140, about 100 to about 120, about 120 to about 200, about 120 to about 180, about 120 to about 160, about 120 to about 140, about 140 to about 200, about 140 to about 180, about 140 to about 160, about 160 to about 200, about 160 to about 180, or about 180 to about 200); performing live-cell single molecule tracking or two-dimensional imaging on about 8 to about 17 cells (e.g., about 10 to about 15, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 cells) in a single field of view; the protein is a protein comprising a mutation (e.g., a mutated protein); the imaged protein comprises a label (e.g., a fluorescent marker or label), an affinity tag (e.g., an affinity tag, His tag (metal ions, such as Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}), biotin (streptavidin), Strep II Tag (Streptavidin or Strep-Tactin), biotin (Streptavidin), calmodulin-binding peptide (CBP) tag (calmodulin), chitin-binding domain (CBD) tag (chitin), maltose-binding protein (MBP) (amylose), glutathione S-transferase (GST) tag (glutathione), S-tag (S-protein of Rnase A), FLAG tag (DYKDDDDK)

(anti-FLAG monoclonal antibod, such as M1, M2, and M5, or a derivative thereof), HaloTag (a reactive chloroalkane linker), SNAP-tag®(e.g., guanine or chloropyrimidine with a benzyl linker to the label)), or a combination thereof; or a combination thereof.

[0077] In any aspect or embodiment described herein, the image protein comprises an affinity tag and a fluorescent label is covalently linked to a ligand of the affinity tag; the method further comprises contacting the cell with a fluorescent label covalently linked to a ligand of an affinity tag, wherein the imaged protein comprises the affinity tag; or a combination thereof.

[0078] In any aspect or embodiment described herein, two-dimensional imaging is performed with a camera (e.g., electron-multiplying charged-coupled device (EMCCD) camera, scientific complementary metal-oxide-semiconductor (sCMOS) camera, or a combination thereof) that has a resolution of about 60 to about 100 nm/pixel (e.g., about 65 to about 95 nm/pixel).

[0079] In any aspect or embodiment described herein, performing live-cell single molecule tracking further comprises preparing a trajectory of localization data points of binding events that comprises: (1) residence time of the imaged protein (i.e., the time of binding to the genome or chromatin), (2) average two-dimensional (XY) position of the imaged protein, (3) the two-dimensional (XY) positions of the imaged protein over successive frames, or (4) a combination thereof; fitting (e.g., 2D Gaussian fitting) individual labels in a frame at a resolution of less than about 35 nanometers (nm) (e.g., less than about 30, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 5 to about 35, about 5 to about 30, about 5 to about 25, about 5 to about 20, about 5 to about 15, about 10 to about 35, about 10 to about 30, about 10 to about 25, about 10 to about 20, about 15 to about 35, about 15 to about 30, about 15 to about 25, about 20 to about 35, about 20 to about 30, or about 25 to about 35 nm); linking localizations in successive frames that are within about 500 nm to form an individual binding trajectory or trajectories; removing genomic and/or chromatic localizations (e.g., the average two-dimensional (XY) position) of the imaged protein that are within about 2 or less microns (e.g., about 0 to about 2 microns) over a period of at least about 8 seconds (e.g., about 8 seconds to about 14 seconds or about 11 seconds); calculating the angle between at least three (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) successive localizations from a residence time window or successive residence time windows (e.g., a window of about 8 to about 20 seconds or about 8 to about 14 seconds, such as 8, 9, 10, 11, 12,

13, 14, 15, 16, 17, 18, 19, or 20 seconds) of the individual binding trajectory or trajectories, wherein each successive residence time window is shifted by about 1, about 2, about 3, about 4, or about 5 seconds (e.g., with a 1 second shift, a first window that is 2 to about 12 seconds of the individual binding trajectory or trajectories, a second window that is about 3 to about 13 seconds of the individual binding trajectory or trajectories, a third window that is about 4 to about 14 seconds of the individual binding trajectory or trajectories, a fourth window that is about 5 to about 15 seconds of the individual binding trajectory or trajectories, a fifth window that is about 6 to about 16 seconds of the individual binding trajectory or trajectories, etc.); or a combination thereof.

[0080] In any aspect or embodiment described herein, calculating the anisotropy formed between three successive localization data points (e.g., calculating the anisotropy formed between three successive localization data points during the trajectory) comprises: determining if the genome and/or chromatin bound imaged protein displays random (e.g., isotropic) or a biased (anisotropic) movement by examining angular measurements (e.g., by graphing the angular measurements, such as on a polar histogram plot) from at least 5 cells (e.g., 5, 6, 7, 8, 9, 10, or more cells) in a given residence window; graphing a plurality of cells (e.g., at least 5 cells, such as 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 cells) in each resident time window on a polar histogram plot; determining the percentage of imaged proteins that move two-dimensionally (e.g., back and forth or oscillatory) with those that only move forward in one dimension in successive frames; quantifying the anisotropic movement as the fold anisotropy (e.g., as the f180/0 ratio, as the percentage of imaged protein that move forward or back compared with imaged proteins that only move forward in successive frames); preparing a time dependent anisotropy profile (e.g., by plotting the fold anisotropy for each residence time window verse the centroid of the residence time window); or a combination thereof.

[0081] In any aspect or embodiment described herein, calculating the anisotropy formed between three successive localization data points comprises: generating a temporal angular signature for a genome and/or chromatin binding event (e.g., one or more, or each, genome and/or chromatin binding event) from one or more cells (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 cells); the temporal angular signature of a genome and/or chromatin binding event comprises, consists essentially of, or consists of, a series of angles after the arrival of the protein onto the genome and/or chromatin (e.g., the first angle of the series of

angles is the arrival of the protein onto the genome and/or chromatin), wherein each angle is calculated from 3 localization data points (e.g., 3 successive localization data points); the temporal angular signature comprises, consists essentially of, or consists of, a residence time window (e.g., a window of about 8 to about 20 seconds or about 8 to about 14 seconds, such as 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 seconds) of the individual binding trajectory or trajectories after the arrival of the protein onto the genome and/or chromatin (e.g., the first angle of the series of angles is the arrival of the protein onto the genome and/or chromatin); quantifying the anisotropic movement of the temporal angular signature for the genome and/or chromatin binding event (e.g., a series of angles after the arrival of the protein onto the genome and/or chromatin) as the fold anisotropy (e.g., as the f180/0 ratio, as the percentage of proteins that move forward or back compared with proteins that only move forward in successive frames); generating a temporal activity profile for a genome and/or chromatin binding event (e.g., one or more, or each, genome and/or chromatin binding event) by plotting the fold anisotropy (e.g., the f180/0 ratio) of the temporal angular signature for the genome and/or chromatin binding event as a function of time (e.g., a function of time after the arrival of the protein onto the genome and/or chromatic); comparing temporal activity profiles from different binding events (e.g., different time periods, residence time windows, successive residence time windows, etc.); or a combination thereof.

[0082] In any aspect or embodiment described herein, the method further comprising: expressing the protein and the imaged protein in the cell (e.g., incubating the cell that expresses the protein and the imaged protein for about 5 hours to about 30 hours, such as under acceptable growth conditions (e.g., about 36 to 38°C at 5%CO_2)); mutating the protein; labeling or tagging the imaged protein (e.g., an affinity tag (such as HaloTag or SNAP-tag®), a fluorescent label, etc.); contacting the cell with a fluorescent label that is covalently linked to a ligand of an affinity tag, wherein the imaged protein comprises the affinity tag; or a combination thereof.

[0083] In any aspect or embodiment described herein, contacting comprises: contacting the cell with the fluorescent label covalently linked to the ligand of the affinity tag is for about 15 to about 30 minutes (e.g., about 15 to about 30, about 15 to about 25, about 15 to 20, about 20 to about 30, about 20 to about 25, of about 25 to about 30 minutes) before performing live-cell single molecule tracking of the imaged protein; contacting the cell with the fluorescent label covalently linked to the ligand of the affinity tag is performed with about 0.5 to about 5.0 nM

(e.g., about 0.5 to about 5.0, about 0.5 to about 4.5, about 0.5 to about 4.0, about 0.5 to about 3.5, about 0.5 to about 3.0, about 0.5 to about 2.5, about 0.5 to about 2.0, about 0.5 to about 1.5, about 1.0 to about 5.0, about 1.0 to about 4.5, about 1.0 to about 4.0, about 1.0 to about 3.5, about 1.0 to about 3.0, about 1.0 to about 2.5, about 1.0 to about 2.0, about 1.5 to about 5.0, about 1.5 to about 4.5, about 1.5 to about 4.0, about 1.5 to about 3.5, about 1.5 to about 3.0, about 1.5 to about 2.5, about 2.0 to about 5.0, about 2.0 to about 4.5, about 2.0 to about 4.0, about 2.0 to about 3.5, about 2.0 to about 3.0, about 2.5 to about 5.0, about 2.5 to about 4.5, about 2.5 to about 4.0, about 2.5 to about 3.5, about 3.0 to about 5.0, about 3.0 to about 4.5, about 3.0 to about 4.0, about 3.5 to about 5.0, about 3.5 to about 4.5, or about 4.0 to about 5.0) of the fluorescent label; or a combination thereof.

[0084] Yet another aspect of the present disclosure provides a method of detecting, examining, and/or screening in a live cell the effect of a modification on a protein that binds or interacts with a genome and/or chromatin, the method comprising: preparing a cell with a wild-type protein; preparing a cell with a modified protein (e.g., a mutated version of the protein or a post translationally modified version of the protein); performing steps (1) and (2) as described herein for (i) the cell with wild-type protein and (ii) the cell with the modified protein.

[0085] In any aspect or embodiment described herein, the method further comprising comparing the anisotropy formed between three successive localization data points (e.g., comparing the anisotropy formed between three successive localization data points during the trajectory) of the wild-type protein and the mutated protein.

[0086] In any aspect or embodiment described herein, the modified protein is a mutated version of the wild-type protein.

[0087] In any aspect or embodiment described herein, preparing the cell with the modified protein comprises mutating the wild-type protein (mutated protein) in a cell.

[0088] In any aspect or embodiment described herein, preparing the cell with the modified protein comprises: obtaining cells from a subject (such as, a hematopoietic stem cell(s) or a cancer cell(s)); growing the obtained cell or cells or replacing the protein of a cell with the protein from the obtained cell or cells; and performing steps (1) and (2) on the obtained cell(s) or the cell(s) with the protein from the obtained cell(s).

[0089] Yet a further aspect of the present disclosure provides a method of detecting, examining, and/or screening a subject for a mutated version of a protein that binds or interacts

with a genome and/or chromatin in a live cell, the method comprising: obtaining a cell with a version of the protein to be examined; performing steps (1) and (2) as described herein for the obtained cell.

[0090] In any aspect or embodiment described herein, the method further comprises comparing the anisotropy formed between three successive localization data points (e.g., comparing the anisotropy formed between three successive localization data points during the trajectory) of the cell with the version of the protein to be examined and a cell comprising a wild-type protein (e.g., a matched cell comprising the wild-type protein).

[0091] Yet an additional aspect of the present disclosure provides a method of detecting, examining, and/or screening in a live cell the effect of an agent on a protein that binds or interacts with a genome and/or chromatin, the method comprising: performing steps (1) and (2) as described herein on (i) a cell with a protein, (ii) a cell with a wild-type protein, (iii) a cell with a modified protein (e.g., mutated version of the protein or a post-translationally modified version of the protein), (iv) the version of the protein to be examined, or (v) a combination thereof; performing steps (1) and (2) on (i) a cell with a protein, (ii) a cell with a wild-type protein, (iii) a cell with a modified protein (e.g., mutated version of the protein or a post translationally modified version of the protein), (iv) the version of the protein to be examined, or (v) a combination thereof, that has been contacted with an agent (e.g., a therapeutic agent, such as a small molecule, a peptide, a protein, siRNA, miRNA, shRNA, antisense RNA, RNAzyme, or DNAzyme, etc.).

[0092] In any aspect or embodiment described herein, the method further comprising: contacting the cell with the agent; calculating the anisotropy or angle formed between three temporally successive localizations during a trajectory of a genomic binding event of the imaged protein in the cell not contacted with the agent and/or a chromatin binding event of the imaged protein in the cell contacted with the agent; and comparing the anisotropy or angle formed between three temporally successive localizations of (i) the imaged protein in the cell not contacted with the agent and having the protein, the wild-type protein, the version of the protein to be examined, and/or the version of the protein to be examined (e.g., mutated version of the protein or the post-translationally modified version of the protein), and (ii) the imaged protein in the cell contacted with the agent and having the protein, the wild-type protein, the version of the protein to be examined, and/or the version of the protein to be examined.

[0093] In any aspect or embodiment described herein, the agent is an inhibitor of the protein is a transcription factor, cyclin-dependent kinase 7 (CDK7), cyclin-dependent kinase 9 (CDK9), xeroderma pigmentosum type B (XPB), transcription factor IIH (TFIIH), enhancer of zeste homolog 2 (EZH2), runt-related transcription factor 1 (RUNX1), nicotinamide adenosine dinucleotide (NAD)-dependent deacetylase sirtuin-1 (SIRT1), or tumor protein P53 (p53).

[0094] In any aspect or embodiment described herein, the imaged protein is an enzyme.

[0095] In any aspect or embodiment described herein, the enzyme is a motor protein, a chromatin remodeling complex (e.g., BRG1 or hbrm-associated factor (BAF) or polybromo-associated BRG1 or Pbrm-associated factor (PBAF)), an RNA polymerase (e.g., RNA polymerase I, II, and/or III), a DNA polymerase (e.g., DNA polymerase β (beta), λ (lamda), σ (sigma), μ (mu), α (alpha), δ (delta), ε (epsilon), η (eta), ι (iota), κ (kappa), Rev1, ζ (zeta), γ (gamma), θ (theta), and/or ν (nu)), CDK-activating kinase (CAK) complex (e.g., CDK7 or CDK9), a telomerase, poly [adenosine diphosphate-ribose] polymerase 1 (PARP1), ribonuclease H1 (RNAse H1), or a reverse transcriptase.

EXAMPLES

[0096] Using live cell single molecule imaging (LcSMI) at slow image acquisition rates (e.g. every about 500 milliseconds), time dependent changes in the anisotropy profiles of four different genomic and/or chromatin bound enzymes under a variety of conditions that both inhibit and potentiate activity were examined. Clear differences in the time dependent anisotropy profiles that correlate with changes in enzyme activity were observed (Figures 3-5, 6C, 6D, and 6E). Clear differences in anisotropy profiles of enzymes that differentially engage wild-type tumor protein P53 (p53) and cancer associated mutant p53 were also observed (Figures 7 and 8). Thus, the analytic method (e.g. time dependent anisotropy of genomic/chromatin bound factors) is a significantly improved technique useful for screening for therapeutics and mutant p53 in cells. The new analytical method has been named genomic bound anisotropy or GEANIS.

[0097] Using LcSMI at slow image acquisition rates (e.g., every about 500 milliseconds), time dependent changes in the anisotropy across individual binding events of two different genomic/chromatin bounds enzymes under a variety of conditions where activity is inhibited or potentiated. Data from the LcSMI experiments in Examples 5 and 6 have been analyzed and demonstrate support that our method can reveal anisotropy differences that vary

over time during a genomic binding event (Figure 9D, RNA Polymerase II) along with small molecule inhibitors (Figures 10A, 10B, 10C, and 10D, RNA Polymerase II) and cellular conditions where metabolite levels have been genetically modified via CRISPR knockout of the nuclear metabolic enzyme, NMNAT1, that generates NAD+ which reduces total cellular NAD+ levels by ~70% (Figures 11A and 11B, poly [adenosine diphosphate-ribose] polymerase 1 (PARP1)). Please note that in general, enzymes that bind for shorter periods of time (e.g. <40 seconds) display reduced temporal fluctuations in anisotropy (Figures 10B and 11A) relative to binding events that last longer than 60 seconds (Figures 10C and 11B).

[0098] Thus, clear differences in the temporal activity anisotropy profiles were observed, which correlate with changes in enzyme activity (Figures 9A-11B). This analytic method (e.g. temporal activity time dependent anisotropy of genomic/chromatin bound factors) provides a significantly improved way to screen for therapeutics and mutant p53 in cell lines and metabolic dysfunction and eventual human patient samples. This new analytical method has been named temporal activity genomic bound anisotropy or taGEANIS.

Materials and Methods of the Examples

[0099] The general method utilized in the examples is described here with the detailed description for each example provided with each example. First, a target protein tagged with a HaloTag or SNAP-tag® was exogenously expressed in tissue culture cells. The tagged proteins were then specifically labeled with commercially fluorescent dyes (e.g., JF549/646-HaloTag Ligand (Promega™, Madison, WI) or SNAP Cell TMR-star/647-SiR (New England Biolabs®, Ipswich, MA)) at a concentration sufficient for single molecule imaging in live cells (about 0.5 to about 5 nM dye for about 15 to about 30 minutes).

[0100] Next, live cells containing fluorescently labeled target proteins were imaged in 2 dimensions at an continuous image acquisition frame rate of 532 milliseconds/frame on an inverted fluorescent microscope (Nikon ECLIPSE T*i*-U (Minato City, Tokyo, Japan) with 100 milliwatt (mW) 532 nanometer (nm) and 100 mW 640 nm Coherent Lasers, and containing an Andor iXon 897 Electron-multiplying charged-coupled device (EMCCD) camera or Teledyne Photometrics PRIME™ 95B scientific complementary metal-oxide-semiconductor (sCMOS) camera capable of detecting single molecules with a resolution ranging from 65 to 95 nm/pixel. The slow image acquisition rate of 532 milliseconds/frame blurs out freely diffusing

fluorescently labeled proteins and is biased towards capturing proteins as they bind and unbind the genome and/or chromatin (Figures 1A and 1B).

[0101] Localizations of individual dye molecules within a given frame were determined via 2D gaussian fitting at less than about 30 nm estimated resolution. Localizations in successive frames that were within about 500 nm are linked together to form an individual binding trajectory that maps the XY position of the genome bound factor over time. Localizations within an individual binding trajectory are averaged to define the average XY position of genome bound factor. A binding trajectory contains 3 key measurements including (1) time of genome binding (e.g. residence time), (2) average XY position of the protein, and (3) XY positions of the protein over successive frames.

[0102] All binding events with a given residence time within an 11 second window (can be varied depending on amount of data being analyzed) beginning with a window at 2-12 seconds were determined. Genome/chromatin bound proteins in this pool of filtered events that are within 2 microns (based on average XY position) were removed to prevent spurious spatial mixing of labeled molecules that may complicate further analysis. Once spatially isolated genome bound proteins are determined, the angle between three successive localizations were calculated throughout the entire binding trajectory (Figure 2A). For example, a binding event that lasts 60 frames (about 30 seconds) will have 58 angular measurements.

[0103] For Examples 1-4 (genome bound anisotropy (GEANIS)), angular measurements from a number of cells (>5 cells) in a given residence time window were then graphed on a polar histogram plot to determine if the genome bound protein displays random (e.g. isotropic) or biased (anisotropic) movement (Figure 2B). Figure 2B shows angular anisotropy histogram plots showing a slight leftward skew of molecules moving forward and back displaying anisotropic movement. Anisotropic movement is quantitatively defined by the fold anisotropy (f180/0 ratio), which reflects the percentage of proteins that move forward and back (e.g. oscillatory) compared with proteins that only move forward in successive frames. In simplified terms, an isotropically bound protein (e.g. Brownian motion) will have a fold anisotropy of 1 with increasing values indicative of a more anisotropic (e.g. oscillatory biased) movement (see, e.g., Figures 2B and 2C). Figure 2C is a schematic showing that an elongating RNA Polymerase II (left) bound to the genome has the potential to generate less anisotropic

movement compared to a paused stationary RNA Polymerase II (right) whose movement is dominated by the oscillatory motion of the genome.

[0104] After determining the anisotropy for all molecules within a given residence time window (e.g. 2-12 seconds), the residence time window for filtering binding events is shifted by 1 second (e.g. 3-13 seconds) and repeat our anisotropy measurements. This process is repeated until there are no longer enough filtered binding events to accurately analyze a given dataset. Finally, the fold anisotropy (f180/0) for a given residence time window is plotted versus the centroid of the residence time window (Figures 3-5, 6B, 7, and 8) to give a time dependent anisotropy profile. Exact details of fluorescent labeling, fluorescent microscope, LcSMI, and image processing can be found below. Software used for XY localization and anisotropy analysis was obtained and modified as noted above from Chen, J., *et al.* Single-molecule dynamics of enhanceosome assembly in embryonic stem cells. *Cell.* 156, 1274-1285 (2014), Kenworthy CA, *et al.* Bromodomains regulate dynamic targeting of the PBAF chromatin remodeling complex to chromatin hubs. *Biophys J.* 121(9):1738-1752 (2022) and Hansen AS, Amitai A, Cattoglio C, Tjian R, Darzacq X. Guided nuclear exploration increases CTCF target search efficiency. Nat Chem Biol. 2020 Mar;16(3):257-266.

[0105] For Examples 5 and 6 (temporal activity genome bound anisotropy (taGEANIS)), a temporal angular signature for each genome binding event is generated. The temporal angular signature consists of the angle between 3 successive XY localizations for an individual genome binding event that is assigned to a time point that corresponds to the time after arrival of the protein onto the genome for the middle XY localization in the 3 localizations that make up the angle (Table 2). For example, if the protein is localized in the first 3 frames after arrival onto the genome (e.g. appearance of a spot) using slow LcSMI (about 0.5 second exposure/frame), the angle between the three XY localizations as occurring about 1 second after arrival of the protein on the genome is assigned. The angle for frames 2,3,4 after protein arrival would be assigned as about 1.5 seconds after arrival of the protein on the genome, etcetera (Table 2). If the temporal angular signature of individual genome binding events within a given 11 second window of residence times (Figure 2a and 2b) were aligned, and the f180/0 ratio vertically at individual time points after arrival of the protein on the genome are calculated (Figure 9C). Then, the f180/0 ratio is plotted as a function of time after arrival of the protein on

the genome (Figure 9D). This temporal activity profile represents how the average anisotropy of individual binding events varies over time during a genome binding event.

Table 2. Illustration of the assignment of angles and calculation of f180/0 to the corresponding time point after the protein (e.g., Pol II) appears on the genome

Time after Pol II Spot Appears (T)	**T=1"**	**T=1.5"**	**T=2.0"**	**T=2.5"**
	Frame 1,2,3	Frame 2,3,4	Frame 3,4,5	Frame 4,5,6
Θ of Pol II Binding Event #1	170°	155°	150°	165°
Θ of Pol II Binding Event #2	150°	60°	162°	30°
Θ of Pol II Binding Event #3	20°	175°	155°	30°
Θ of Pol II Binding Event #4	26°	30°	17°	19°
Fold Anisotropy (f180/0)	(2/2) = 1	(2/1) = 2	(3/1) = 3	(1/3) = 0.33

[0106] After determining the anisotropy for all molecules within a given residence time window (e.g. 2-12 seconds), the residence time window for filtering binding events is shifted by 1 second (e.g. 3-13 seconds) and repeat our anisotropy measurements. This process is repeated until there are no longer enough filtered binding events to accurately analyze a given dataset. Finally, the temporal activity profile from different windows of residence times was compared to determine how the movement differs amongst the same protein that binds the genome for different lengths of time (Figures 10B and 10C). Exact details of fluorescent labeling, fluorescent microscope, LcSMI, and image processing can be found below. Software used for XY localization and anisotropy analysis was obtained and modified as noted below from Chen, J., *et al.* Single-molecule dynamics of enhanceosome assembly in embryonic stem cells. *Cell.* 156, 1274-1285 (2014), Kenworthy CA, *et al.* Bromodomains regulate dynamic targeting of the PBAF chromatin remodeling complex to chromatin hubs. *Biophys J.* 121(9):1738-1752 (2022),

and Hansen AS, Amitai A, Cattoglio C, Tjian R, Darzacq X. Guided nuclear exploration increases CTCF target search efficiency. Nat Chem Biol. 2020 Mar;16(3):257-266.

[0107] Briefly, for the Examples, U2OS cells containing Halo-tagged or SNAP-tagged proteins were grown at 37°C with 5% CO_2 to a density of about 5 x 10^5 cells on 35 mm MatTek imaging dishes in complete Dulbecco's Modified Eagle Medium (high glucose Dulbecco's Modified Eagle Medium (DMEM), 10% FBS, 2 mM Glutamax (Fisher Scientific), 100 I.U./mL Penicillin, and 100 microg/mL Streptomycin (Corning)). Immediately prior to imaging, cells were incubated with 10 nM SNAP-Cell 647-SiR (New England Biolabs) and/or 0.4 nM JF549-HTL at 37°C for a total of 30 and 15 minutes, respectively. Cells were then washed three times with 1X phosphate-buffered saline (PBS), replaced with complete DMEM and further incubated for 30 minutes at 37°C to remove unincorporated dye. Cells were then washed two times with 1X PBS and placed in L-15 imaging media (Life technologies) + 10% FBS for imaging.

[0108] Fluorescent molecules were illuminated via HILO using a 532 nm laser (13 W/cm^2) and a 640 nm laser (9.5 W/cm^2). Time-lapse two-dimensional (2D) images of single molecules were acquired at 25°C or 37°C with a customized inverted Nikon Eclipse Ti microscope with a 100× oil-immersion objective lens (1.49 NA, Nikon, Melville, NY) and were further magnified about 1.7-1.9× post-objective. Images were acquired at 2 Hz for about 9-18 minutes using an EMCCD (iXon, Andor, Belfast, UK) with a 512 × 512 pixel field of view (final pixel size of 84-95 nm) or a Prime95b sCMOS (Photometrix) with a 1200 x 1200 pixel field of view (final pixel size of 65-95 nm). Imaging at 25°C is preferred since there is less cell movement and microscope drift at this temperature compared to acquisition of images at 37°C. Cell movement and microscope drift can introduce background movement of fluorescent signals that are unrelated to a protein's anisotropy when bound to the genome.

[0109] Movies of acquired images were processed to subtract background in ImageJ using a rolling ball radius of 50 pixels. Background subtracted movies were subjected to multi-target tracking to resolve the trajectories of individual molecule using a GUI-based implementation, SLIMfast as described in Chen J *et al.* Cell 2014. Localization of individual molecules was achieved by fitting point spread functions (PSFs) of discrete single spots with a 2D Gaussian function. Tracking of single-molecule chromatin-binding events was performed by connecting protein XY localizations between consecutive frames. Tracking was based upon a maximum expected diffusion constant of 0.05 μm^2/s and allowed for 1.5 s gaps in trajectories

due to blinking or missed localizations. Once the XY positions of molecules in individual trajectories are determined, we use a custom in house Matlab software suite to group the temporal XY positions of molecules in binding trajectories along with their residence times in individual cells under a specific condition (e.g. treatment) along with metadata into a single file (e.g. 5-30 cells/file). This file is then further processed to filter for molecules containing a window of residence times for further processing. These filtered molecules (e.g. binding trajectories) are then further spatially filtered (no closer than 2 microns) and used to determine the angles between three successive frames and the f180/0 values using custom in house designed Matlab software that is modified from Matlab software originally described in reference: Hansen AS, Amitai A, Cattoglio C, Tjian R, Darzacq X. Guided nuclear exploration increases CTCF target search efficiency. Nat Chem Biol. 2020 Mar;16(3):257-266.

[0110] Once the f180/0 values are determined for molecules within a window residence times, we use additional custom in house Matlab software to plot the data shown in the figures.

Example 1. Mutational Inactivation of a Polymerase.

[0111] Anisotropy measurements were taken of SNAP-tagged wild-type DNA Polymerase δ (upper line) or catalytically dead DNA Polymerase δ (lower line) binding events in human LOX cells. Binding events were filtered using 13 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis). Figure 3 shows the LcSMI data. The data of Figure 3 demonstrates that the method of the present disclosure can reveal anisotropy differences between active and inactive enzymes. Mutational inactivation leads to a general elevation of anisotropy values over time. In general, as described in greater detail below, it was observed that enzymes inhibited via mutational inactivation or small molecule inhibitors, typically display an overall elevated anisotropy profile compared to wild-type or untreated enzyme (Figures 3, 5, 6C, 6D, and 6E).

Example 2. Mutational Activation of a Polymerase.

[0112] A single point mutant (E1126G and its homologous mutation E1103G in yeast RNA Polymerase II) has previously been shown to increase RNA Polymerase II elongation rates by >1.4 fold and have a distinct mechanism of transcription initiation compared to wild-type RNA Polymerase II. Anisotropy measurements were taken of wild-type RNA Polymerase II/SNAP-RPB1 (Figure 4, upper line) or fast E1126G mutant RNA Polymerase II/SNAP-RPB1 (Figure 4, lower line) in U2OS cells. Binding events were filtered using 11 second windows of

residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis). Figure 4 shows that acceleration of RNA Polymerase II elongation rates via mutation results in an overall decrease in the anisotropy profile over time. The LcSMI data in Figure 4 demonstrates that the method of the present disclosure can reveal anisotropy differences between an active enzyme (RNA Polymerase II) and a mutant enzyme (fast E1126G mutant RNA Polymerase II) with enhanced activity. The data of Figure 4 demonstrates that enzymes activated via mutations or small molecules, display an overall reduced anisotropy profile compared to wild-type or untreated enzyme.

Example 3. Screening for Efficacy of Therapeutics.

[0113] Small molecule inhibitors THZ1 (cyclin-dependent kinase 7 (CDK7) inhibitor), Triptolide (xeroderma pigmentosum type B (XPB)-transcription factor IIH (TFIIH) inhibitor), and Flavopiridol (cyclin-dependent kinase 9 (CDK9) inhibitor) are well established and target different factors that are known to regulate distinct functional steps associated with RNA Polymerase II mediated transcription initiation/elongation. Anisotropy measurements were taken of wild-type RNA Polymerase II/SNAP-RPB1 after treatment with dimethyl sulfoxide/control, Flavopiridol/CDK9 inhibitor, THZ1/CDK7 inhibitor, and Triptolide/XPB inhibitor in U2OS cells. Binding events were filtered using 11 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis). The LcSMI data in Figure 5 demonstrate the practical use of the method of the present disclosure for screening small molecule inhibitors. Figure 5 shows that each of the inhibitors cause different effects on the time-dependent fold anisotropy profiles of fluorescently labeled SNAP-tagged RNA Polymerase II.

[0114] For example, Triptolide is known to prevent RNA Polymerase II escape from the promoter by blocking the ATPase activity of XPB, which is required for maximal deoxyribonucleic acid (DNA) strand separation and promoter escape. The data shows a nearly identical anisotropy profile from 7 to 41 seconds with Triptolide and the control. However, the number of RNA Polymerase II binding events lasting over 41 seconds is drastically reduced in the presence of Triptolide, truncating the anisotropy profile relative to control samples consistent with a prior fluorescent recovery after photobleaching (FRAP) based imaging study (Steurer, B., *et al.* Live-cell analysis of endogenous GFP-RPB1 uncovers rapid turnover of initiating and promoter-paused RNA Polymerase II. *Proceedings of the National Academy of*

Sciences of the United States of America 115, E4368-E4376 (2018)). By inhibiting XPB, RNA Polymerase II is unable to escape the promoter and effectively remains bound to the genome past a certain time point (e.g., about 41 seconds). This boundary for promoter escape (about 41 seconds) is consistent with previous studies showing a transition point at about 42 to 60 seconds related to RNA Polymerase II elongating into the gene body (Steurer, B., *et al.* Live-cell analysis of endogenous GFP-RPB1 uncovers rapid turnover of initiating and promoter-paused RNA Polymerase II. *Proceedings of the National Academy of Sciences of the United States of America* 115, E4368-E4376 (2018); Darzacq, X., *et al.* In vivo dynamics of RNA polymerase II transcription. *Nature Structural & Molecular Biology* 14, 796-806 (2007)).

[0115] THZ1 inhibits the CDK7 subunit of TFIIH, which acts on RNA Polymerase II early (within 2.4 seconds) in the formation of the transcription pre-initiation complex. Figure 5 confirms that THZ1 acts within seconds (< 6 seconds) after RNA Polymerase II loads onto the genome with an immediate deviation from the anisotropy profile of the control.

[0116] CDK7 is required to activate CDK9, which acts after the RNA Polymerase II escapes the promoter (e.g., greater than 42 seconds after RNA Polymerase II loads onto the genome). Remarkably, as shown in Figure 5, the anisotropy profiles of THZ1 (CDK7 inhibitor) and Flavopiridol (CDK9 inhibitor) are nearly identical starting at approximately 40 seconds after the RNA Polymerase II loads onto the genome. This suggests a common mechanism regulating RNA Polymerase II between 40 to 60 seconds after the polymerase binds the genome, consistent with the known dependencies between CDK7 and CDK9. The data demonstrates that the method of the present disclosure can be used to distinguish the effects of different therapeutics that specifically target a variety of enzymes that regulate transcription initiation.

[0117] Polybromo-associated BRG1 or Pbrm-associated factor (PBAF) is a multi-subunit chromatin remodeling complex that both repositions and evicts nucleosomes via adenosine triphosphate (ATP) hydrolysis. Two key subunits within PBAF are polybromo (BAF180) and SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4 or BRG1). PBAF interacts with DNA packaging protein Histone H3 with an acetylated histone tail (H3K14ac). Currently, the arrangement of the BAF180 bromodomains (BD) 1-6 and the BRG1 BD are speculative. The inventors of this disclosure are investigating the structure of the PBAF complex containing polybromo

(BAF180) and BRG1 bound to a nucleosome (pdb: 7VDV) with and without PFI3 treatment. It has been shown that the small molecule PFI3 targets the BRG1 BD and inhibits its binding to H3K14ac on the nucleosome. Inventors have also investigated the structure of the PBAF complex bound to a nucleosome (7VDV) of BD5 mutant (N739F) PBAF with and without PFI3 treatment. They determined that BD5 mutations also weaken the activity of PBAF, likely due to disruption of the BD2, BD4, and BD5 network that functions collaboratively to enhance H3K14ac association. Finally, disruption of both BRG1 and BD5 results in a low anisotropy, which reflects an increase in the unregulated activity of PBAF (data not shown). See Table 1 above regarding *in vitro* binding activity of BRG1 bromodomains for H3K14ac.

[0118] Table 3 shows a Grid Analysis of Halo-BAF180 containing all six BDs deleted (BD Δ) or inactivation of the BRG1 bromodomain with the PFI3 small molecule (PFI3) compared to wild-type (WT). The Grid Analysis was able to parse out four distinct populations for all three conditions, with the most dynamic population denoted P_1 (% of molecules) and stable populations denoted P_2 – P_4. Residence times for corresponding populations are denoted with τ.

Table 3. Grid Analysis of Halo-BAF180 containing all six BDs deleted (BDΔ) or inactivation of the BRG1 bromodomain (PFI3) compared to wild-type (WT)

	PBAF	**P1 (%)**	**τ1 (s)**	**P2 (%)**	**τ2 (s)**	**P3 (%)**	**τ3 (s)**	**P4 (%)**	**τ4 (s)**
Halo-BAF180 Wild type	WT	68.8 ± 0.2	0.505 ± 0.001	22.4 ± 0.1	3.18 ± 0.02	6.63 ± 0.12	11.9 ± 0.3	2.1 ± 0.1	42.6 ± 1.5
Halo-BAF180 BD1-6Δ	BDΔ	76.9 ± 0.3	0.476 ± 0.004	18.6 ± 0.3	3.06 ± 0.03	3.94 ± 0.11	13.0 ± 0.4	0.55 ± 0.09	43.3 ± 3.4
Halo-BAF180	PFI3	71.0 ± 0.5	0.485 ± 0.004	21.4 ± 0.5	3.00 ± 0.08	6.15 ± 0.48	9.9 ± 0.6	1.53 ± 0.14	42.4 ± 2.5

Wild type + PFI3									

[0119] Mutations in the BRG1-Associated Factor 180 (BAF180) subunit of the ATP-dependent polybromo-associated BAF (PBAF) chromatin remodeling complex are associated with about 40% of clear cell renal cell carcinoma (ccRCC) patients. Figure 6A is a domain schematic of BAF180 and BRG1. Individual BAF180 bromodomains (BDs) differentially interact with DNA packaging protein Histone H3 with an acetylated histone tail (H3K14ac)-containing nucleosomes *in vitro*. The arrangement of the six bromodomains of BAF180 (BD1-6) and BRG1 Bromodomain are speculative. Figure 6B illustrates an anisotropy analysis schematic. The genome fluctuates like a harpsichord. Therefore, the activity of PBAF can be assessed by measuring the propensity of Halo-BAF180 to recoil back over three successive frames. Stalled PBAF will have a strong directional bias (higher f180/0), thereby reflecting a complex with less activity. Active PBAF will have less directional bias and have a lower f180/0 value.

[0120] Figure 6C shows anisotropy measurements taken of wild-type PBAF, Halo-BAF180 WT control and PFI3 treated along with mutant PBAF containing 6 bromodomains deleted Halo-BAF180 ΔBD in U2OS cells. Binding events were filtered using 11 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis). Figure 6C demonstrates that the treatment of cells with PFI3, which targets the bromodomain of the BRG1 subunit of PBAF13, results in an overall elevated anisotropy of Halo-tagged BAF180 compared to the control. The anisotropy profile after PFI3 treatment was distinct from the profile after removing the six bromodomains of BAF180 (Figure 6C). The data demonstrates that the method of the present disclosure can reveal distinct changes in PBAF activity when different subunits are inhibited or mutated.

[0121] Table 4 shows a Grid Analysis of Halo-BAF180 containing point mutations in distinct BDs. The Grid Analysis was able to parse out five distinct populations for BAF180 containing a mutation in BD3 (N463F), while detecting four populations for all other conditions.

Table 4. Grid Analysis of Halo-BAF180 containing point mutations in distinct BDs

	PBAF	P1 (%)	τ1 (s)	P2 (%)	τ2 (s)	P3 (%)	τ3 (s)	P4 (%)	τ4 (s)	P5 (%)	τ5 (s)
Halo-BAF180 Wild type + DMSO	WT	68.8 ± 0.2	0.505 ± 0.001	22.4 ± 0.1	3.18 ± 0.02	6.63 ± 0.12	11.9 ± 0.3	2.1 ± 0.1	42.6 ± 1.5		
Halo-BAF180 N463F + DMSO	N463F	68.2 ± 0.8	0.515 ± 0.007	19.7 ± 0.8	2.86 ± 0.11	8.00 ± 1.80	8.8 ± 0.7	2.02 ± 0.88	23.1 ± 5.1	1.66 ± 0.46	56.5 ± 5.1
Halo-BAF180 N739F + DMSO	N739F	71.8 ± 0.7	0.505 ± 0.004	20.2 ± 0.7	3.07 ± 0.11	5.24 ± 0.71	9.6 ± 1.2	2.75 ± 0.46	29.9 ± 2.9		
Halo-BAF180 N739F + PFI3	N739F	73.3 ± 0.6	0.478 ± 0.006	19.2 ± 0.5	2.93 ± 0.09	6.28 ± 0.48	10.4 ± 0.6	1.22 ± 0.17	47.6 ± 3.4		

[0122] Anisotropy of BAF180 containing distinct point mutations that inactivate acetyl-lysine binding is shown in Figure 6D. Mutation of BD5 (N739F) causes PBAF to stall and be less active, whereas mutation of BD3 (N463F) causes PBAF to be more active. These results agree with experiments performed *in vitro*, where BD5 was found to enhance PBAF's association with H3K14ac-containing nucleosomes, while BD3 represses binding. Anisotropy of BAF180 containing a BD5 mutation with and without BRG1 BD inhibition is shown in Figure 6E. As shown in Figure 6E, the N739F mutation causes BAF180 to become less active. However, additional inhibition of the BRG1 bromodomain results in substantial decreases in

anisotropy. These changes reflects a complex communication between the BAF180 and BRG1 BDs. BD5 mutations weaken the activity of PBAF, likely due to disruption of the BD2, BD4, and BD5 network that functions collaboratively to enhance H3K14ac association. Finally, disruption of both BRG1 and BD5 results in a low anisotropy, which reflects an increase in the unregulated activity of PBAF.

Example 4. Screening for Cancer Mutations in p53 and Therapeutics Targeting Cancer Hot Spot Mutant p53.

[0123] Over 50% of cancer patients contain a mutant tumor protein P53 or cellular tumor antigen p53 (p53) with over 20% of these patients harboring hot spot mutations in three residues (namely, R175, R248, and R273). Prior structural work by the inventors of the present disclosure demonstrated that p53 binds RNA Polymerase II in a region that associates with transcription factor IIH (TFIIH) to regulate promoter escape and elongation. Anisotropy measurements were taken of wild-type RNA polymerase II/SNAP-RPB1 after induction of wild-type p53 or cancer mutant p53 R273H in U2OS cells. A control lacking induction of p53 was also examined. Binding events were filtered using 11 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis). The LcSMI data of Figure 7 demonstrates that the methods of the present disclosure can be used to screen cells for hot spot mutations in p53. Briefly, time-dependent anisotropy profiles of RNA Polymerase II were measured after p53 induction in the presence of wild-type p53 or cancer mutant p53 R273H, which were further compared with wild-type p53 without induction. The data of Figure 7 demonstrates that there are very distinct differences in RNA Polymerase II anisotropy profiles starting at approximately 35 seconds after RNA Polymerase II loads onto the genome with wild-type and mutant p53 having opposing effects. This time frame (about 30 to about 41 seconds) is associated with TFIIH's ability to separate the DNA strands and facilitate promoter escape (see, e.g., Figure 5). Interestingly, it was previously shown that wild-type p53 inhibits RNA Polymerase II elongation *in vivo*. Furthermore, it was previously shown that mutant p53 R273H does not inhibit RNA Polymerase II elongation and does not inhibit XPB. Thus, the data disclosed herein is consistent with wild-type p53 binding to RNA Polymerase II and inhibiting TFIIH's activity *in vivo*. Therefore, the method of the present disclosure can differentially discern the presence of wild-type p53 versus cancer hot spot mutant p53 in cells.

[0124] Clonal hematopoiesis of indeterminate potential (CHIP) is often the prelude to hematological malignancies including leukemia. A prior study has shown that CHIP is associated with the ability of enhancer of zeste homolog 2 (EZH2), a histone methylase that targets histone H3 protein with tri-methylation of lysine 27 (H3K27me3), to selectively interact with mutant p53 R248W compared to wild-type p53. Expression of mutant p53 in hematopoietic stem and progenitor cell (HSPC) populations results in increased levels of H3K27me3, suggesting enhanced activity of EZH2 in these cells.

[0125] Therefore, being able to easily screen for cells containing mutant p53 R248W versus wild-type p53 would be beneficial for early intervention efforts to prevent progression of CHIP to leukemia in patients. Anisotropy measurements were taken of Halo-tagged EZH2 after induction of wild-type p53 or cancer mutant p53 R248W in U2OS cells. Binding events were filtered using 11 second windows of residence times, each window shifted by 1 second (centroid of residence time window listed on the x-axis). The LcSMI data in Figure 8 demonstrates a clear difference in anisotropy profiles of Halo-tagged EZH2 in osteocarcinoma cells (U2OS) with wild-type p53 versus mutant p53 R248W. Thus, mutant p53 R248W may differentially interact with EZH2 as compared to wild-type p53 in cancer cells. The data also implies a conserved mechanism of mutant p53 altering EZH2 activity in cancers.

[0126] The method of the present disclosure was able to differentially discern the presence of wild-type p53 and cancer mutant p53 in cells through the examination of two different targets—RNA Polymerase II in Figure 7 and EZH2 in Figure 8. Thus, the method of the present disclosure can be used as a screening system based on a series of U2OS cell lines containing SNAP-tagged RNA Polymerase II or Halo-tagged EZH2 with the ability to induce expression of wild-type p53 or cancer associated hot spot mutant p53 (such as, R175H, R248W or R273H). These cell lines would be combined with LcSMI and GEANIS to screen for compounds that selectively disrupt the activity of cancer hot spot mutant p53 proteins versus wild-type p53.

Example 5. Screening for Efficacy of Therapeutics

[0127] Data from the following LcSMI experiments have been analyzed and demonstrate practical uses for screening of small molecule inhibitors. Please note that each of the small molecule inhibitors (Triptolide, XPB-TFIIH inhibitor. And NVP-1, CDK9 inhibitor) examined are well established and target different factors that are known to regulate distinct

functional steps associated with Pol II mediated transcription initiation/elongation. The temporal activity profile of fluorescently labeled SNAP-tagged Pol II with Triptolide (XPB-TFIIH inhibitor) is consistent with GEANIS data showing very little effect of Triptolide compared to a control on Pol II binding events lasting 30-40 seconds. This indicates that TFIIH/XPB activity is not present on a Pol II binding event lasting less than 40 seconds (Figure 10A). In the absence of TFIIH/XPB activity, Pol II is unable to escape the promoter (Figure 10A) and effectively gets unloaded from the genome after about 40 seconds. This boundary for promoter escape (about 40 seconds) is consistent with previous studies showing a transition point at about 42 to about 60 seconds related to Pol II elongating into the gene body.

[0128] The inhibitor of CDK9 (NVP-1) is predicted to act on Pol II that escapes the promoter, which was predicted to occur roughly about 42 to about 60 seconds after Pol II arrives on the genome. The taGEANIS temporal activity profile of Pol II shows a very strong fluctuation of anisotropy at about 85 to about 90 seconds after Pol II arrival on the genome (Figure 10C). Furthermore, this temporal fluctuation in anisotropy at about 85 to about 90 seconds is significantly enhanced and sustained in the presence of NVP-1 (Figure 10D). This unique temporal activity quantification allows one to further discriminate inhibitors that mechanistically act on different steps of the kinetic reaction profile of enzymes/proteins. Overall, the temporal activity genomic bound anisotropy (taGEANIS) method can be used to distinguish the effects of different therapeutics that specifically target a variety of enzymes that regulate transcription initiation.

Example 6. Screening for Metabolic Dysfunction in Cells

[0129] Data from the following LcSMI experiments have been analyzed and demonstrate practical uses for screening for metabolic dysfunction in cells. Data indicate that knocking out nicotinamide nucleotide adenylyltransferase 1 (NMNAT1), the nuclear metabolic enzyme that generates oxidized nicotinamide adenine dinucleotide (NAD+), reduces total cellular NAD+ levels by roughly 70%. Reduced cellular NAD+ levels are also a hallmark of ageing. Poly [adenosine diphosphate-ribose] polymerase 1 (PARP1) is an enzyme that absolutely requires NAD+ for its activity in DNA repair and Pol II pause release. The data shows that there are minimal NAD+ dependent temporal fluctuations in anisotropy for PARP1 binding events lasting 30-40 seconds (Figure 11A) compared to longer lived (60-70 seconds) PARP1 binding events (Figure 11B). This data suggests that (1) temporal fluctuations in

anisotropy in PARP1 during long lived binding events (e.g., 60-70 seconds) are numerous and particularly dependent on NAD+, and (2) temporal fluctuations in anisotropy for short lived PARP1 genomic binding events (<40 seconds) are relatively insensitive to NAD+ levels in cells and therefore likely represent binding events with reduced PARP1 enzymatic activity. Overall, this unique temporal activity quantification of PARP1 activity allows one to discriminate metabolic dysfunction related to varying NAD+ levels in cells.

[0130] While several embodiments of the invention of the present disclosure have been shown and described herein, it will be understood that such embodiments are provided by way of example only. Numerous variations, changes and substitutions will occur to those skilled in the art without departing from the spirit of the invention. Rather, the present disclosure is to cover all modifications, equivalents, and alternatives falling within the scope of the present disclosure as defined by the following appended claims and their legal equivalents. Accordingly, it is intended that the description and appended claims cover all such variations as fall within the spirit and scope of the invention.

[0131] The contents of all references, patents, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

[0132] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims. It is understood that the detailed examples and embodiments described herein are given by way of example for illustrative purposes only, and are in no way considered to be limiting to the invention. Various modifications or changes in light thereof will be suggested to persons skilled in the art and are included within the spirit and purview of this application and are considered within the scope of the appended claims. For example, the relative quantities of the ingredients can be varied to optimize the desired effects, additional ingredients can be added, and/or similar ingredients can be substituted for one or more of the ingredients described. Additional advantageous features and functionalities associated with the systems, methods, and processes of the present invention will be apparent from the appended claims. Moreover, those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

What Is Claimed Is:

1. A method of detecting, examining, and/or screening activity of a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin in a binding event that occurs in a live cell, the method comprising:

(a) performing live-cell single molecule tracking of the protein (e.g., an enzyme) in a binding event bound to the genome and/or chromatin of the cell at an image acquisition frame rate of about 300 to about 700 milliseconds/frame (e.g., about 400 to about 600 milliseconds/frame or about 500 milliseconds/frame) to generate 3 or more localization data points of binding events in the cell and from the 3 or more localization data points; and

(b) calculating the anisotropy formed between three successive localization data points.

2. The method of claim 1, wherein performing live-cell single molecule tracking comprises:

performing two-dimensional imaging (e.g., fluorescent microscopy);

performing live-cell single molecule tracking at about 25°C to about 37°C (e.g. about 25°C to about 37°C, about 25°C to about 36°C, about 25°C to about 35°C, about 25°C to about 34°C, about 25°C to about 33°C, about 25°C to about 32°C, about 25°C to about 31°C, about 25°C to about 30°C, about 25°C to about 29°C, about 25°C to about 28°C, about 25°C to about 27°C, about 26°C to about 37°C, about 26°C to about 36°C, about 26°C to about 35°C, about 26°C to about 34°C, about 26°C to about 33°C, about 26°C to about 32°C, about 26°C to about 31°C, about 26°C to about 30°C, about 26°C to about 29°C, about 26°C to about 28°C, about 27°C to about 37°C, about 27°C to about 36°C, about 27°C to about 35°C, about 27°C to about 34°C, about 27°C to about 33°C, about 27°C to about 32°C, about 27°C to about 31°C, about 27°C to about 30°C, about 27°C to about 29°C, about 28°C to about 37°C, about 28°C to about 36°C, about 28°C to about 35°C, about 28°C to about 34°C, about 28°C to about 33°C, about 28°C to about 32°C, about 28°C to about 31°C, about 28°C to about 30°C, about 29°C to about 37°C, about 29°C to about 36°C, about 29°C to about 35°C, about 29°C to about 34°C, about 29°C to about 33°C, about 29°C to about 32°C, about 29°C to about 31°C, about 30°C to about 37°C, about 30°C to about 36°C, about 30°C to about 35°C, about 30°C to about 34°C, about

30°C to about 33°C, about 30°C to about 32°C, about 31°C to about 37°C, about 31°C to about 36°C, about 31°C to about 35°C, about 31°C to about 34°C, about 31°C to about 33°C, about 32°C to about 37°C, about 32°C to about 36°C, about 32°C to about 35°C, about 32°C to about 34°C, about 33°C to about 37°C, about 33°C to about 36°C, about 33°C to about 35°C, about 34°C to about 37°C, about 34°C to about 36°C, or about 35°C to about 37°C);

performing live-cell single molecule tracking for up to about 10 minutes (e.g., about 1 to about 10, about 1 to about 9, about 1 to about 8, about 1 to about 7, about 1 to about 6, about 1 to about 5, about 1 to about 4, about 1 to about 3, about 1 to about 2, about 2 to about 10, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4, about 2 to about 3, about 3 to about 10, about 3 to about 9, about 3 to about 8, about 3 to about 7, about 3 to about 6, about 3 to about 5, about 3 to about 4, about 4 to about 10, about 4 to about 9, about 4 to about 8, about 4 to about 7, about 4 to about 6, about 4 to about 5, about 5 to about 10, about 5 to about 9, about 5 to about 8, about 5 to about 7, about 5 to about 6, about 6 to about 10, about 6 to about 9, about 6 to about 8, about 6 to about 7, about 7 to about 10, about 7 to about 9, about 7 to about 8, about 8 to about 10, about 8 to about 9, or about 9 to about 10 minutes);

performing live-cell single molecule tracking includes illuminating the cell for about 200 to about 700 millisecond (e.g., about 200 to about 700, about 200 to about 600, about 200 to about 500, about 200 to about 400, about 200 to about 300, about 300 to about 700, about 300 to about 600, about 300 to about 500, about 300 to about 400, about 400 to about 700, about 400 to about 600, about 400 to about 500, about 500 to about 700, about 500 to about 600, or about 600 to about 700) every about 1 to about 4 seconds (e.g., about 1 to about 4, about 1 to about 3.5, about 1 to about 3, about 1 to about 2.5, about 1 to about 2, about 1 to about 1.5, about 1.5 to about 4, about 1.5 to about 3.5, about 1.5 to about 3, about 1.5 to about 2.5, about 1.5 to about 2, about 2 to about 4, about 2 to about 3.5, about 2 to about 3, about 2 to about 2.5, about 2.5 to about 4, about 2.5 to about 3.5, about 2.5 to about 3, about 3 to about 4, about 3 to about 3.5, or about 3.5 to about 4 seconds);

performing live-cell single molecule tracking on about 8 to about 17 cells (e.g., about 10 to about 15, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 cells) in a single field of view;

the protein is a protein comprising a mutation (e.g., a mutated protein or mutated enzyme);

the protein comprises a label (e.g., a fluorescent marker or label), an affinity tag (e.g., an affinity tag, His tag (metal ions, such as Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}), biotin (streptavidin), Strep II Tag (Streptavidin or Strep-Tactin), biotin (Streptavidin), calmodulin-binding peptide (CBP) tag (calmodulin), chitin-binding domain (CBD) tag (chitin), maltose-binding protein (MBP) (amylose), glutathione S-transferase (GST) tag (glutathione), S-tag (S-protein of RNase A), FLAG tag (DYKDDDDK) (anti-FLAG monoclonal antibody, such as M1, M2, and M5, or a derivative thereof), HaloTag (a reactive chloroalkane linker), SNAP-tag®(e.g., guanine or chloropyrimidine with a benzyl linker to the label)), or a combination thereof; or

a combination thereof.

3. The method of claim 2, wherein:

the protein comprises an affinity tag and a fluorescent label is covalently linked to a ligand of the affinity tag;

the method further comprises contacting the cell with a fluorescent label covalently linked to a ligand of an affinity tag, wherein the protein comprises the affinity tag; or

a combination thereof.

4. The method of claim 2 or claim 3, wherein two-dimensional imaging is performed with a camera (e.g., electron-multiplying charged-coupled device (EMCCD) camera, scientific complementary metal-oxide-semiconductor (sCMOS) camera, or a combination thereof) that has a resolution of about 60 to about 100 nm/pixel (e.g., about 65 to about 95 nm/pixel).

5. The method of any one of claims 1-4, wherein performing live-cell single molecule tracking further comprises preparing a trajectory of localization data points of binding events that comprises:

(1) residence time of the protein (i.e., the time of binding to the genome or chromatin), (2) average two-dimensional (XY) position of the protein, (3) the two-dimensional (XY) positions of the protein over successive frames, or (4) a combination thereof;

fitting (e.g., 2D Gaussian fitting) individual labels in a frame at a resolution of less than about 35 nanometers (nm) (e.g., less than about 30, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 5 to about 35, about 5 to about 30, about 5 to about 25, about 5 to about 20, about 5 to about 15, about 10 to about 35, about 10 to about 30, about 10 to about 25, about 10 to about 20, about 15

to about 35, about 15 to about 30, about 15 to about 25, about 20 to about 35, about 20 to about 30, or about 25 to about 35 nm);

linking localizations in successive frames that are within about 500 nm to form an individual binding trajectory or trajectories;

removing genomic and/or chromatic localizations (e.g., the average two-dimensional (XY) position) of the protein that are within about 2 or less microns (e.g., about 0 to about 2 microns) over a period of at least about 8 seconds (e.g., about 8 seconds to about 14 seconds or about 11 seconds);

calculating the angle between at least three (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) successive localizations from a residence time window or successive residence time windows (e.g., a window of about 8 to about 20 seconds or about 8 to about 14 seconds, such as 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 seconds) of the individual binding trajectory or trajectories, wherein each successive residence time window is shifted by about 1, about 2, about 3, about 4, or about 5 seconds (e.g., with a 1 second shift, a first window that is 2 to about 12 seconds of the individual binding trajectory or trajectories, a second window that is about 3 to about 13 seconds of the individual binding trajectory or trajectories, a third window that is about 4 to about 14 seconds of the individual binding trajectory or trajectories, a fourth window that is about 5 to about 15 seconds of the individual binding trajectory or trajectories, a fifth window that is about 6 to about 16 seconds of the individual binding trajectory or trajectories, etc.); or

a combination thereof.

6. The method of any one of claims 1-5, wherein calculating the anisotropy formed between three successive localization data points comprises:

determining if the genome and/or chromatin bound protein displays random (e.g., isotropic) or a biased (anisotropic) movement by examining angular measurements (e.g., by graphing the angular measurements, such as on a polar histogram plot) from at least 5 cells (e.g., 5, 6, 7, 8, 9, 10, or more cells) in a given residence window;

graphing a plurality of cells (e.g., at least 5 cells, such as 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 cells) in each resident time window on a polar histogram plot;

determining the percentage of proteins that move two-dimensionally (e.g., back and forth or oscillatory) with those that only move forward in one dimension in successive frames;

quantifying the anisotropic movement as the fold anisotropy (e.g., as the f180/0 ratio, as the percentage of proteins that move forward or back compared with proteins that only move forward in successive frames);

preparing a time dependent anisotropy profile (e.g., by plotting the fold anisotropy for each residence time window verse the centroid of the residence time window); or

a combination thereof.

7. The method of any one of claims 1-5, wherein calculating the anisotropy formed between three successive localization data points comprises:

generating a temporal angular signature for a genome and/or chromatin binding event (e.g., one or more, or each, genome and/or chromatin binding event);

the temporal angular signature of a genome and/or chromatin binding event comprises, consists essentially of, or consists of, a series of angles after the arrival of the protein onto the genome and/or chromatin (e.g., the first angle of the series of angles is the arrival of the protein onto the genome and/or chromatin), wherein each angle is calculated from 3 localization data points (e.g., 3 successive localization data points);

the temporal angular signature comprises, consists essentially of, or consists of, a residence time window (e.g., a window of about 8 to about 20 seconds or about 8 to about 14 seconds, such as 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 seconds) of the individual binding trajectory or trajectories after the arrival of the protein onto the genome and/or chromatin (e.g., the first angle of the series of angles is the arrival of the protein onto the genome and/or chromatin);

quantifying the anisotropic movement of the temporal angular signature for the genome and/or chromatin binding event (e.g., a series of angles after the arrival of the protein onto the genome and/or chromatin) as the fold anisotropy (e.g., as the f180/0 ratio, as the percentage of proteins that move forward or back compared with proteins that only move forward in successive frames);

generating a temporal activity profile for a genome and/or chromatin binding event (e.g., one or more, or each, genome and/or chromatin binding event) by plotting the fold anisotropy (e.g., the f180/0 ratio) of the temporal angular signature for the genome and/or chromatin binding event as a function of time (e.g., a function of time after the arrival of the protein onto the genome and/or chromatic);

comparing temporal activity profiles from different binding events (e.g., different time periods, residence time windows, successive residence time windows, etc.); or

a combination thereof.

8. The method of any one of claims 1-7, further comprising

expressing the protein in the cell (e.g., incubating the cell that expresses the protein for about 5 hours to about 30 hours, such as under acceptable growth conditions (e.g., about 36 to 37°C at 5%CO_2));

mutating the protein;

labeling or tagging the protein (e.g., an affinity tag (such as HaloTag or SNAP-tag®), a fluorescent label, etc.);

contacting the cell with a fluorescent label that is covalently linked to a ligand of an affinity tag, wherein the protein comprises the affinity tag; or

a combination thereof.

9. The method of claim 8, wherein contacting comprises:

contacting the cell with the fluorescent label covalently linked to the ligand of the affinity tag is for about 15 to about 30 minutes (e.g., about 15 to about 30, about 15 to about 25, about 15 to 20, about 20 to about 30, about 20 to about 25, of about 25 to about 30 minutes) before performing live-cell single molecule tracking of the protein;

contacting the cell with the fluorescent label covalently linked to the ligand of the affinity tag is performed with about 0.5 to about 5.0 nM (e.g., about 0.5 to about 5.0, about 0.5 to about 4.5, about 0.5 to about 4.0, about 0.5 to about 3.5, about 0.5 to about 3.0, about 0.5 to about 2.5, about 0.5 to about 2.0, about 0.5 to about 1.5, about 1.0 to about 5.0, about 1.0 to about 4.5, about 1.0 to about 4.0, about 1.0 to about 3.5, about 1.0 to about 3.0, about 1.0 to about 2.5, about 1.0 to about 2.0, about 1.5 to about 5.0, about 1.5 to about 4.5, about 1.5 to about 4.0, about 1.5 to about 3.5, about 1.5 to about 3.0, about 1.5 to about 2.5, about 2.0 to about 5.0, about 2.0 to about 4.5, about 2.0 to about 4.0, about 2.0 to about 3.5, about 2.0 to about 3.0, about 2.5 to about 5.0, about 2.5 to about 4.5, about 2.5 to about 4.0, about 2.5 to about 3.5, about 3.0 to about 5.0, about 3.0 to about 4.5, about 3.0 to about 4.0, about 3.5 to about 5.0, about 3.5 to about 4.5, or about 4.0 to about 5.0) of the fluorescent label; or

a combination thereof.

10. A method of detecting, examining, and/or screening in a live cell the effect of a modification on a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin, the method comprising:

preparing a cell with a wild-type protein;

preparing a cell with a modified protein (e.g., a mutated version of the protein or a post-translationally modified version of the protein); and

performing steps (a) and (b) of any one of claims 1-9 for (i) the cell with wild-type protein and (ii) the cell with the modified protein.

11. The method of claim 10, further comprising comparing the anisotropy formed between three successive localization data points of the wild-type protein and the mutated protein.

12. The method of claim 10 or claim 11, wherein the modified protein is a mutated version of the wild-type protein.

13. The method of any one of claims 10-12, wherein preparing the cell with the modified protein comprises mutating the wild-type protein (mutated protein or mutated enzyme) in a cell.

14. The method of claim 10 or claim 11, wherein preparing the cell with the modified protein:

obtaining cells from a subject (such as, a hematopoietic stem cell(s) or a cancer cell(s));

growing the obtained cell or cells or replacing the protein of a cell with the protein from the obtained cell or cells; and

performing steps (a) and (b) on the obtained cell(s) or the cell(s) with the protein from the obtained cell(s).

15. A method of detecting, examining, and/or screening a subject for a mutated version of a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin in a live cell, the method comprising:

obtaining a cell with a version of the protein to be examined; and

performing steps (a) and (b) of any one of claims 1-9 for the obtained cell.

16. The method of claim 15, further comprising comparing the anisotropy formed between three successive localization data points of the binding event with the version of the

protein to be examined and a cell comprising a wild-type protein (e.g., a matched cell comprising the wild-type protein or enzyme).

17. A method of detecting, examining, and/or screening in a live cell the effect of an agent on a protein (e.g., an enzyme) that binds or interacts with a genome and/or chromatin, the method comprising:

performing steps (a) and (b) of any one of claims 1-16 on (i) a cell with a protein (e.g., an enzyme), (ii) a cell with a wild-type protein (e.g., a wild-type enzyme), (iii) a cell with a version of the protein to be examined (e.g., mutated version of the protein or enzyme or a post-translationally modified version of the protein or enzyme), (iv) the version of the protein (e.g., enzyme) to be examined, or (v) a combination thereof; and

performing steps (a) and (b) on (i) a cell with a protein (e.g., an enzyme), (ii) a cell with a wild-type protein (e.g., wild-type enzyme), (iii) a cell with the modified protein (e.g., mutated version of the protein or enzyme or a post-translationally modified version of the protein or enzyme), (iv) the version of the protein (e.g., enzyme) to be examined, or (v) a combination thereof, that has been contacted with an agent (e.g., a therapeutic agent, such as a small molecule, a peptide, a protein, siRNA, miRNA, shRNA, antisense RNA, RNAzyme, or DNAzyme, etc.).

18. The method of claim 17, further comprising:

contacting the cell with the agent;

calculating the anisotropy or angle formed between three temporally successive localizations during a trajectory of a genomic binding event of the protein, wild-type protein, the version of the protein to be examined, and/or mutated protein in the cell contacted with the agent and/or a chromatin binding event of the protein, wild-type protein, and/or mutated protein in the cell contacted with the agent; and

comparing the anisotropy or angle formed between three temporally successive localizations of

(i) the protein, the wild-type protein, the version of the protein to be examined, and/or the modified protein (e.g., mutated version of protein or enzyme or a post-translationally modified version of the protein or enzyme) in the cell not contacted with the agent, and

(ii) the protein, the wild-type protein, the version of the protein to be examined, and/or the modified protein (e.g., mutated version of protein or enzyme or a post-translationally modified version of the protein or enzyme) in the cell contacted with the agent.

19. The method of claim 17 or claim 18, wherein the agent is an inhibitor of a chromatin remodeling complex (e.g., BRG1 or hbrm-associated factor (BAF) or polybromo-associated BRG1 or Pbrm-associated factor (PBAF)), an RNA polymerase (e.g., RNA polymerase I, II, and/or III), a DNA polymerase (e.g., DNA polymerase β (beta), λ (lamda), σ (sigma), μ (mu), α (alpha), δ (delta), ε (epsilon), η (eta), ι (iota), κ (kappa), Rev1, ζ (zeta), γ (gamma), θ (theta), and/or ν (nu)), a telomerase, or a reverse transcriptase.

20. The method of any one of claims 1-19, wherein the protein, the wild-type protein, the version of the protein to be examined, and/or the modified protein is an enzyme.

21. A method of detecting, examining, and/or screening activity of a protein that binds or interacts with a genome and/or chromatin in a binding event that occurs in a live cell, the method comprising:

(1) performing live-cell single molecule tracking of an imaged protein (e.g., an imaged enzyme) in a binding event that interacts with the protein and is bound to the genome and/or chromatin of the cell at an image acquisition frame rate of about 300 to about 700 milliseconds/frame (e.g., about 400 to about 600 milliseconds/frame or about 500 milliseconds/frame) to generate 3 or more localization data points of binding events in the cells; and

(2) calculating the anisotropy formed between three successive localization data points,

wherein the anisotropy of the imaged protein is indicative of the interaction of the protein with the genome and/or chromatin.

22. The method of claim 21, wherein performing live-cell single molecule tracking comprises:

performing two-dimensional imaging (e.g., fluorescent microscopy);

performing live-cell single molecule tracking at about 25°C to about 37°C (e.g. about 25°C to about 37°C, about 25°C to about 36°C, about 25°C to about 35°C, about 25°C to about 34°C, about 25°C to about 33°C, about 25°C to about 32°C, about 25°C to about 31°C, about 25°C to about 30°C, about 25°C to about 29°C, about 25°C to about 28°C, about 25°C to about 27°C, about 26°C to about 37°C, about 26°C to about 36°C, about 26°C to about 35°C, about

26°C to about 34°C, about 26°C to about 33°C, about 26°C to about 32°C, about 26°C to about 31°C, about 26°C to about 30°C, about 26°C to about 29°C, about 26°C to about 28°C, about 27°C to about 37°C, about 27°C to about 36°C, about 27°C to about 35°C, about 27°C to about 34°C, about 27°C to about 33°C, about 27°C to about 32°C, about 27°C to about 31°C, about 27°C to about 30°C, about 27°C to about 29°C, about 28°C to about 37°C, about 28°C to about 36°C, about 28°C to about 35°C, about 28°C to about 34°C, about 28°C to about 33°C, about 28°C to about 32°C, about 28°C to about 31°C, about 28°C to about 30°C, about 29°C to about 37°C, about 29°C to about 36°C, about 29°C to about 35°C, about 29°C to about 34°C, about 29°C to about 33°C, about 29°C to about 32°C, about 29°C to about 31°C, about 30°C to about 37°C, about 30°C to about 36°C, about 30°C to about 35°C, about 30°C to about 34°C, about 30°C to about 33°C, about 30°C to about 32°C, about 31°C to about 37°C, about 31°C to about 36°C, about 31°C to about 35°C, about 31°C to about 34°C, about 31°C to about 33°C, about 32°C to about 37°C, about 32°C to about 36°C, about 32°C to about 35°C, about 32°C to about 34°C, about 33°C to about 37°C, about 33°C to about 36°C, about 33°C to about 35°C, about 34°C to about 37°C, about 34°C to about 36°C, or about 35°C to about 37°C);

performing live-cell single molecule tracking for up to about 10 or 20 minutes (e.g., about 1 to about 10, about 1 to about 9, about 1 to about 8, about 1 to about 7, about 1 to about 6, about 1 to about 5, about 1 to about 4, about 1 to about 3, about 1 to about 2, about 2 to about 10, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4, about 2 to about 3, about 3 to about 10, about 3 to about 9, about 3 to about 8, about 3 to about 7, about 3 to about 6, about 3 to about 5, about 3 to about 4, about 4 to about 10, about 4 to about 9, about 4 to about 8, about 4 to about 7, about 4 to about 6, about 4 to about 5, about 5 to about 10, about 5 to about 9, about 5 to about 8, about 5 to about 7, about 5 to about 6, about 6 to about 10, about 6 to about 9, about 6 to about 8, about 6 to about 7, about 7 to about 10, about 7 to about 9, about 7 to about 8, about 8 to about 10, about 8 to about 9, or about 9 to about 10 minutes);

performing live-cell single molecule tracking includes illuminating the cell for about 200 to about 700 millisecond (e.g., about 200 to about 700, about 200 to about 600, about 200 to about 500, about 200 to about 400, about 200 to about 300, about 300 to about 700, about 300 to about 600, about 300 to about 500, about 300 to about 400, about 400 to about 700,

about 400 to about 600, about 400 to about 500, about 500 to about 700, about 500 to about 600, or about 600 to about 700) every about 1 to about 4 seconds (e.g., about 1 to about 4, about 1 to about 3.5, about 1 to about 3, about 1 to about 2.5, about 1 to about 2, about 1 to about 1.5, about 1.5 to about 4, about 1.5 to about 3.5, about 1.5 to about 3, about 1.5 to about 2.5, about 1.5 to about 2, about 2 to about 4, about 2 to about 3.5, about 2 to about 3, about 2 to about 2.5, about 2.5 to about 4, about 2.5 to about 3.5, about 2.5 to about 3, about 3 to about 4, about 3 to about 3.5, or about 3.5 to about 4 seconds);

performing live-cell single molecule tracking on about 8 to about 17 cells (e.g., about 10 to about 15, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 cells) in a single field of view;

the protein is a protein comprising a mutation (e.g., a mutated protein);

the imaged protein comprises a label (e.g., a fluorescent marker or label), an affinity tag (e.g., an affinity tag, His tag (metal ions, such as Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}), biotin (streptavidin), Strep II Tag (Streptavidin or Strep-Tactin), biotin (Streptavidin), calmodulin-binding peptide (CBP) tag (calmodulin), chitin-binding domain (CBD) tag (chitin), maltose-binding protein (MBP) (amylose), glutathione S-transferase (GST) tag (glutathione), S-tag (S-protein of RNase A), FLAG tag (DYKDDDDK) (anti-FLAG monoclonal antibod, such as M1, M2, and M5, or a derivative thereof), HaloTag (a reactive chloroalkane linker), SNAP-tag®(e.g., guanine or chloropyrimidine with a benzyl linker to the label)), or a combination thereof; or

a combination thereof.

23. The method of claim 22, wherein:

the imaged protein comprises an affinity tag and a fluorescent label is covalently linked to a ligand of the affinity tag;

the method further comprises contacting the cell with a fluorescent label covalently linked to a ligand of an affinity tag, wherein the imaged protein comprises the affinity tag; or

a combination thereof.

24. The method of claim 22 or claim 23, wherein two-dimensional imaging is performed with a camera (e.g., electron-multiplying charged-coupled device (EMCCD) camera, scientific complementary metal-oxide-semiconductor (sCMOS) camera, or a combination thereof) that has a resolution of about 60 to about 100 nm/pixel (e.g., about 65 to about 95 nm/pixel).

25. The method of any one of claims 21-24, wherein performing live-cell single molecule tracking further comprises preparing a trajectory of localization data points of binding events that comprises:

(1) residence time of the imaged protein (i.e., the time of binding to the genome or chromatin), (2) average two-dimensional (XY) position of the imaged protein, (3) the two-dimensional (XY) positions of the imaged protein over successive frames, or (4) a combination thereof;

fitting (e.g., 2D Gaussian fitting) individual labels in a frame at a resolution of less than about 35 nanometers (nm) (e.g., less than about 30, about 1 to about 35, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 5 to about 35, about 5 to about 30, about 5 to about 25, about 5 to about 20, about 5 to about 15, about 10 to about 35, about 10 to about 30, about 10 to about 25, about 10 to about 20, about 15 to about 35, about 15 to about 30, about 15 to about 25, about 20 to about 35, about 20 to about 30, or about 25 to about 35 nm);

linking localizations in successive frames that are within about 500 nm to form an individual binding trajectory or trajectories;

removing genomic and/or chromatic localizations (e.g., the average two-dimensional (XY) position) of the imaged protein that are within about 2 or less microns (e.g., about 0 to about 2 microns) over a period of at least about 8 seconds (e.g., about 8 seconds to about 14 seconds or about 11 seconds);

calculating the angle between at least three (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) successive localizations from a residence time window or successive residence time windows (e.g., a window of about 8 to about 20 seconds or about 8 to about 14 seconds, such as 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 seconds) of the individual binding trajectory or trajectories, wherein each successive residence time window is shifted by about 1, about 2, about 3, about 4, or about 5 seconds (e.g., with a 1 second shift, a first window that is 2 to about 12 seconds of the individual binding trajectory or trajectories, a second window that is about 3 to about 13 seconds of the individual binding trajectory or trajectories, a third window that is about 4 to about 14 seconds of the individual binding trajectory or trajectories, a fourth window that is about 5 to about 15 seconds of the individual binding trajectory or trajectories, a fifth window that is about 6 to about 16 seconds of the individual binding trajectory or trajectories, etc.); or

a combination thereof.

26. The method of any one of claims 21-25, wherein calculating the anisotropy formed between three successive localization data points comprises:

determining if the genome and/or chromatin bound imaged protein displays random (e.g., isotropic) or a biased (anisotropic) movement by examining angular measurements (e.g., by graphing the angular measurements, such as on a polar histogram plot) from at least 5 cells (e.g., 5, 6, 7, 8, 9, 10, or more cells) in a given residence window;

graphing a plurality of cells (e.g., at least 5 cells, such as 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 cells) in each resident time window on a polar histogram plot;

determining the percentage of the imaged proteins that move two-dimensionally (e.g., back and forth or oscillatory) with those that only move forward in one dimension in successive frames;

quantifying the anisotropic movement as the fold anisotropy (e.g., as the f180/0 ratio, as the percentage of imaged proteins that move forward or back compared with imaged proteins that only move forward in successive frames);

preparing a time dependent anisotropy profile (e.g., by plotting the fold anisotropy for each residence time window verse the centroid of the residence time window); or

a combination thereof.

27. The method of any one of claims 21-25, wherein calculating the anisotropy formed between three successive localization data points comprises:

generating a temporal angular signature for a genome and/or chromatin binding event (e.g., one or more, or each, genome and/or chromatin binding event);

the temporal angular signature of a genome and/or chromatin binding event comprises, consists essentially of, or consists of, a series of angles after the arrival of the protein onto the genome and/or chromatin (e.g., the first angle of the series of angles is the arrival of the protein onto the genome and/or chromatin), wherein each angle is calculated from 3 localization data points (e.g., 3 successive localization data points);

the temporal angular signature comprises, consists essentially of, or consists of, a residence time window (e.g., a window of about 8 to about 20 seconds or about 8 to about 14 seconds, such as 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 seconds) of the individual binding trajectory or trajectories after the arrival of the protein onto the genome and/or

chromatin (e.g., the first angle of the series of angles is the arrival of the protein onto the genome and/or chromatin);

quantifying the anisotropic movement of the temporal angular signature for the genome and/or chromatin binding event (e.g., a series of angles after the arrival of the protein onto the genome and/or chromatin) as the fold anisotropy (e.g., as the f180/0 ratio, as the percentage of proteins that move forward or back compared with proteins that only move forward in successive frames);

generating a temporal activity profile for a genome and/or chromatin binding event (e.g., one or more, or each, genome and/or chromatin binding event) by plotting the fold anisotropy (e.g., the f180/0 ratio) of the temporal angular signature for the genome and/or chromatin binding event as a function of time (e.g., a function of time after the arrival of the protein onto the genome and/or chromatic);

comparing temporal activity profiles from different binding events (e.g., different time periods, residence time windows, successive residence time windows, etc.); or

a combination thereof.

28. The method of any one of claims 21-26, further comprising

expressing the protein and the imaged protein in the cell (e.g., incubating the cell that expresses the protein and the imaged protein for about 5 hours to about 30 hours, such as under acceptable growth conditions (e.g., about 36 to 38°C at 5%CO_2));

mutating the protein;

labeling or tagging the imaged protein (e.g., an affinity tag (such as HaloTag or SNAP-tag®), a fluorescent label, etc.);

contacting the cell with a fluorescent label that is covalently linked to a ligand of an affinity tag, wherein the imaged protein comprises the affinity tag; or

a combination thereof.

29. The method of claim 28, wherein contacting comprises:

contacting the cell with the fluorescent label covalently linked to the ligand of the affinity tag is for about 15 to about 30 minutes (e.g., about 15 to about 30, about 15 to about 25, about 15 to 20, about 20 to about 30, about 20 to about 25, of about 25 to about 30 minutes) before performing live-cell single molecule tracking of the imaged protein;

contacting the cell with the fluorescent label covalently linked to the ligand of the affinity tag is performed with about 0.5 to about 5.0 nM (e.g., about 0.5 to about 5.0, about 0.5 to about 4.5, about 0.5 to about 4.0, about 0.5 to about 3.5, about 0.5 to about 3.0, about 0.5 to about 2.5, about 0.5 to about 2.0, about 0.5 to about 1.5, about 1.0 to about 5.0, about 1.0 to about 4.5, about 1.0 to about 4.0, about 1.0 to about 3.5, about 1.0 to about 3.0, about 1.0 to about 2.5, about 1.0 to about 2.0, about 1.5 to about 5.0, about 1.5 to about 4.5, about 1.5 to about 4.0, about 1.5 to about 3.5, about 1.5 to about 3.0, about 1.5 to about 2.5, about 2.0 to about 5.0, about 2.0 to about 4.5, about 2.0 to about 4.0, about 2.0 to about 3.5, about 2.0 to about 3.0, about 2.5 to about 5.0, about 2.5 to about 4.5, about 2.5 to about 4.0, about 2.5 to about 3.5, about 3.0 to about 5.0, about 3.0 to about 4.5, about 3.0 to about 4.0, about 3.5 to about 5.0, about 3.5 to about 4.5, or about 4.0 to about 5.0) of the fluorescent label; or

a combination thereof.

30. A method of detecting, examining, and/or screening in a live cell the effect of a modification on a protein that binds or interacts with a genome and/or chromatin, the method comprising:

preparing a cell with a wild-type protein;

preparing a cell with a modified protein (e.g., a mutated version of the protein or a post translationally modified version of the protein); and

performing steps (1) and (2) of any one of claims 21-29 for (i) the cell with wild-type protein and (ii) the cell with the modified protein.

31. The method of claim 30, further comprising comparing the anisotropy formed between three successive localization data points of the wild-type protein and the mutated protein.

32. The method of claim 30 or claim 31, wherein the modified protein is a mutated version of the wild-type protein.

33. The method of any one of claims 30-32, wherein preparing the cell with the modified protein comprises mutating the wild-type protein (mutated protein) in a cell.

34. The method of claim 30 or claim 31, wherein preparing the cell with the modified protein comprises:

obtaining cells from a subject (such as, a hematopoietic stem cell(s) or a cancer cell(s));

growing the obtained cell or cells or replacing the protein of a cell with the protein from the obtained cell or cells; and

performing steps (1) and (2) on the obtained cell(s) or the cell(s) with the protein from the obtained cell(s).

35. A method of detecting, examining, and/or screening a subject for a mutated version of a protein that binds or interacts with a genome and/or chromatin in a live cell, the method comprising:

obtaining a cell with a version of the protein to be examined; and

performing steps (1) and (2) of any one of claims 21-29 for the obtained cell.

36. The method of claim 35, further comprising comparing the anisotropy formed between three successive localization data points of the cell with the version of the protein to be examined and a cell comprising a wild-type protein (e.g., a matched cell comprising the wild-type protein).

37. A method of detecting, examining, and/or screening in a live cell the effect of an agent on a protein that binds or interacts with a genome and/or chromatin, the method comprising:

performing steps (1) and (2) of any one of claims 21-36 on (i) a cell with a protein, (ii) a cell with a wild-type protein, (iii) a cell with a modified protein (e.g., mutated version of the protein or a post-translationally modified version of the protein), (iv) the version of the protein to be examined, or (v) a combination thereof; and

performing steps (1) and (2) on (i) a cell with a protein, (ii) a cell with a wild-type protein, (iii) a cell with a modified protein (e.g., mutated version of the protein or a post translationally modified version of the protein), (iv) the version of the protein to be examined, or (v) a combination thereof, that has been contacted with an agent (e.g., a therapeutic agent, such as a small molecule, a peptide, a protein, siRNA, etc.).

38. The method of claim 37, further comprising:

contacting the cell with the agent;

calculating the anisotropy or angle formed between three temporally successive localizations during a trajectory of a genomic binding event of the imaged protein in the cell not contacted with the agent and/or a chromatin binding event of the imaged protein in the cell contacted with the agent; and

comparing the anisotropy or angle formed between three temporally successive localizations of

(i) the imaged protein in the cell not contacted with the agent and having the protein, the wild-type protein, the version of the protein to be examined, and/or the version of the protein to be examined (e.g., mutated version of the protein or the post-translationally modified version of the protein), and

(ii) the imaged protein in the cell contacted with the agent and having the protein, the wild-type protein, the version of the protein to be examined, and/or the version of the protein to be examined.

39. The method of claim 37 or claim 38, wherein the agent is an inhibitor of cyclin-dependent kinase 7 (CDK7), cyclin-dependent kinase 9 (CDK9), xeroderma pigmentosum type B (XPB), transcription factor IIH (TFIIH), enhancer of zeste homolog 2 (EZH2), or tumor protein P53 (p53).

40. The method of any one of claim 20-38, wherein the protein is a transcription factor, cyclin-dependent kinase 7 (CDK7), cyclin-dependent kinase 9 (CDK9), xeroderma pigmentosum type B (XPB), enhancer of zeste homolog 2 (EZH2), runt-related transcription factor 1 (RUNX1), nicotinamide adenosine dinucleotide (NAD)-dependent deacetylase sirtuin-1 (SIRT1), or tumor protein P53 (p53).

41. The method of any one of claims 21-40, wherein the imaged protein is an enzyme.

42. The method of claim 20 or 41, wherein the enzyme is a motor protein, a chromatin remodeling complex (e.g., BRG1 or hbrm-associated factor (BAF) or polybromo-associated BRG1 or Pbrm-associated factor (PBAF)), an RNA polymerase (e.g., RNA polymerase I, II, and/or III), a DNA polymerase (e.g., DNA polymerase β (beta), λ (lamda), σ (sigma), μ (mu), α (alpha), δ (delta), ε (epsilon), η (eta), ι (iota), κ (kappa), Rev1, ζ (zeta), γ (gamma), θ (theta), and/or ν (nu)), CDK-activating kinase (CAK) complex (e.g., CDK7 or CDK9), a telomerase, poly [adenosine diphosphate-ribose] polymerase 1 (PARP1), ribonuclease H1 (RNAse H1), or a reverse transcriptase.

FIG. 1A

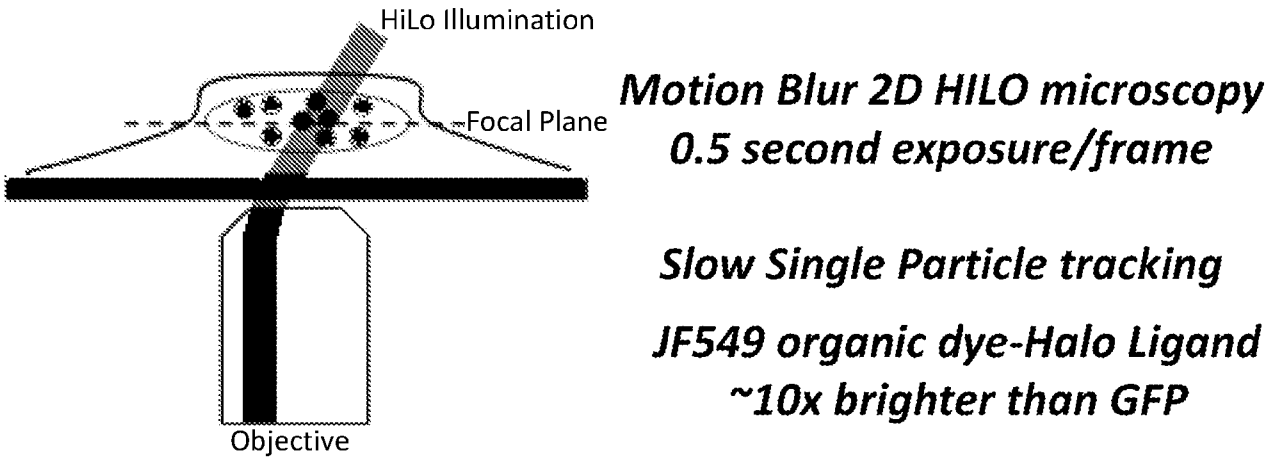

HiLo Illumination
Focal Plane
Objective
Motion Blur 2D HILO microscopy
0.5 second exposure/frame
Slow Single Particle tracking
JF549 organic dye-Halo Ligand
~10x brighter than GFP

FIG. 1B

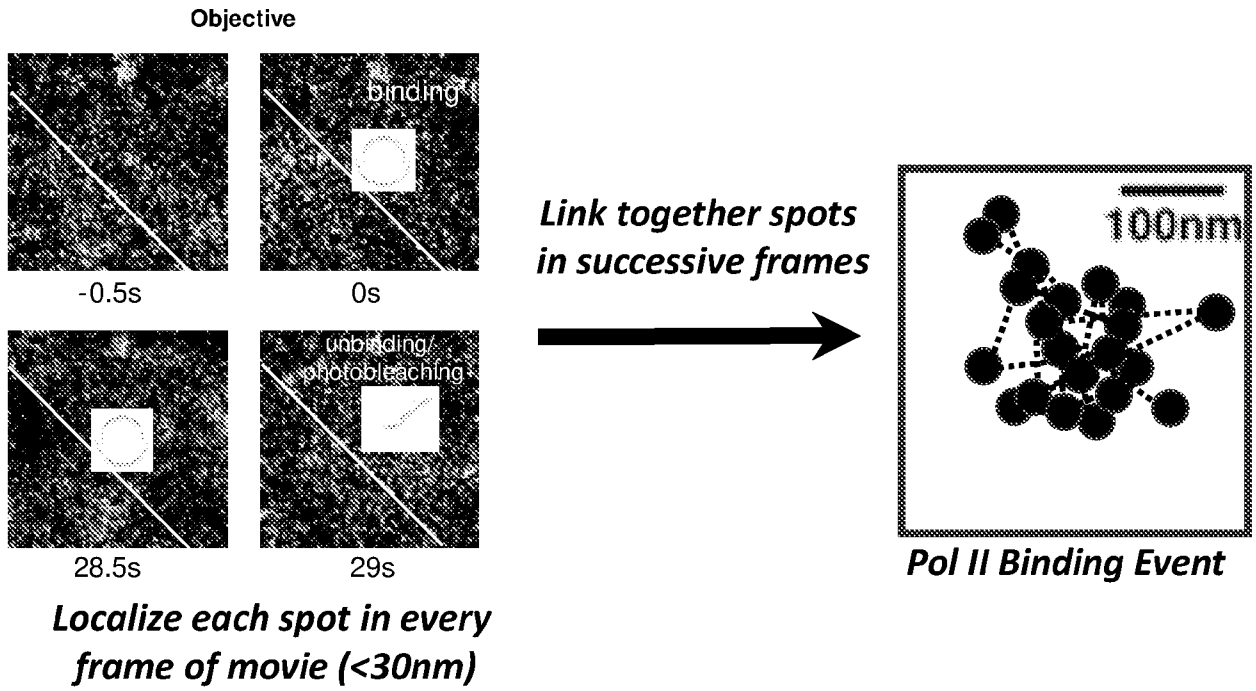

Objective
binding
-0.5s
0s
unbinding/
photobleaching
28.5s
29s
Localize each spot in every
frame of movie (<30nm)
Link together spots
in successive frames
100nm
Pol II Binding Event

FIG. 2A

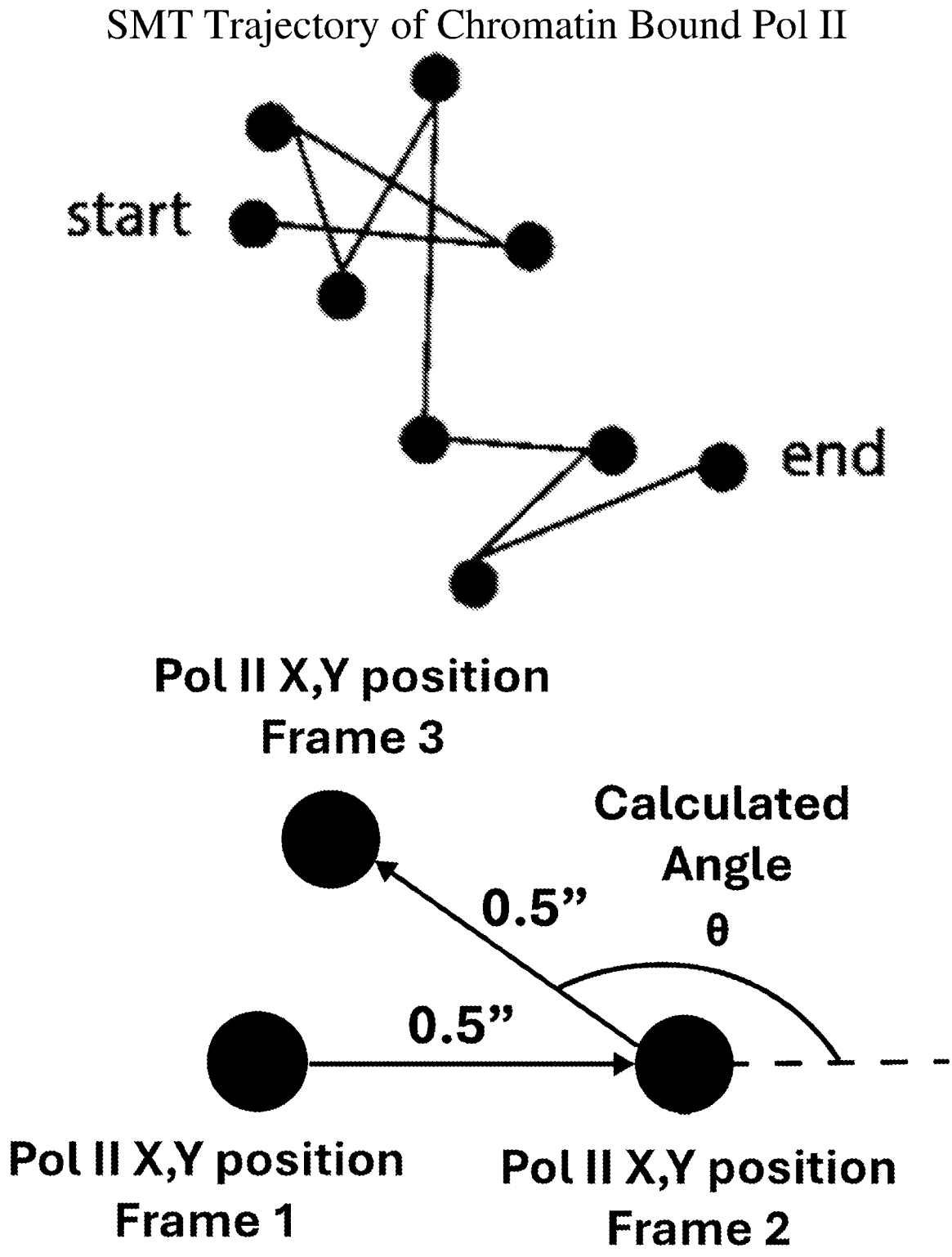
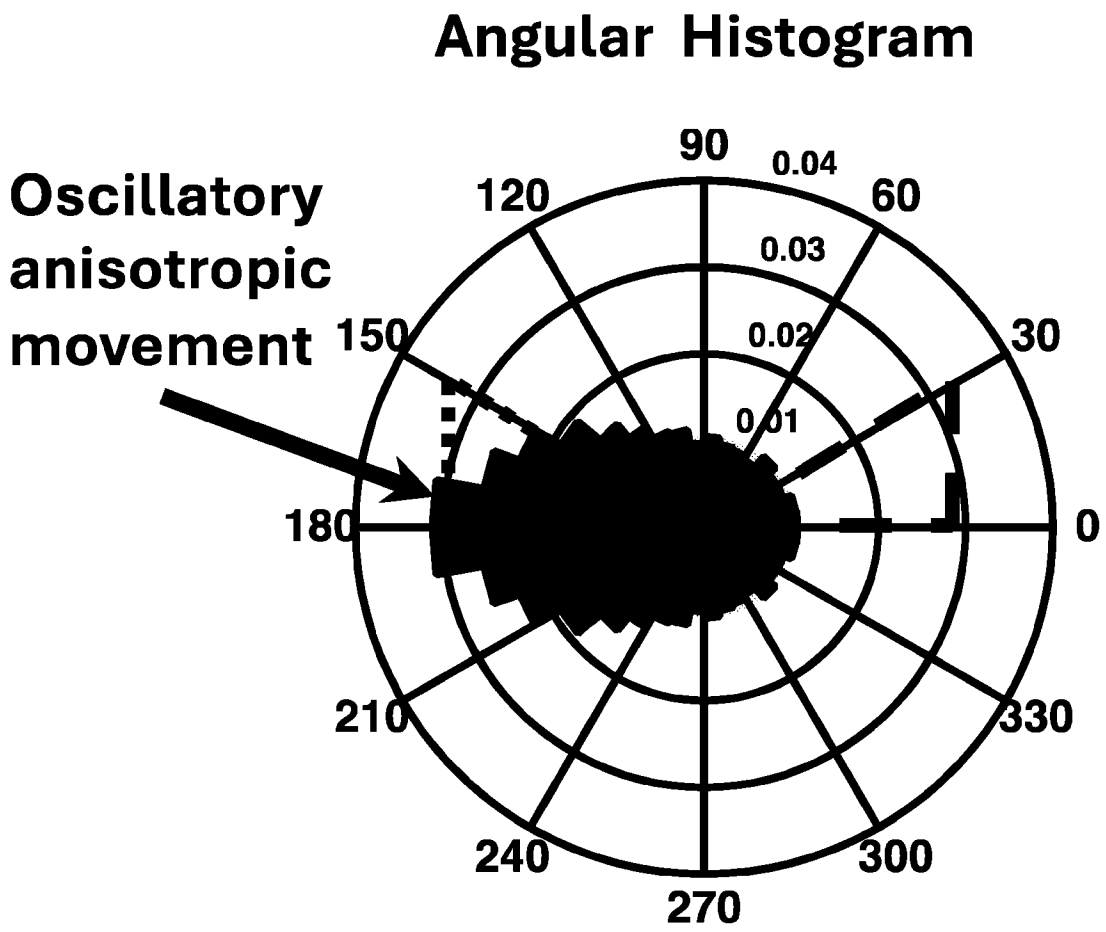

SMT Trajectory of Chromatin Bound Pol II
start
end
Pol II X,Y position
Frame 3
Calculated
Angle
θ
0.5”
0.5”
Pol II X,Y position
Frame 1
Pol II X,Y position
Frame 2

FIG. 2B



Fold Anisotropy (f180/0)

# of Restricted Movements (150-180°)

# of Normalization Movements (0-30°)

Brownian Motion = Fold Anisotropy of 1

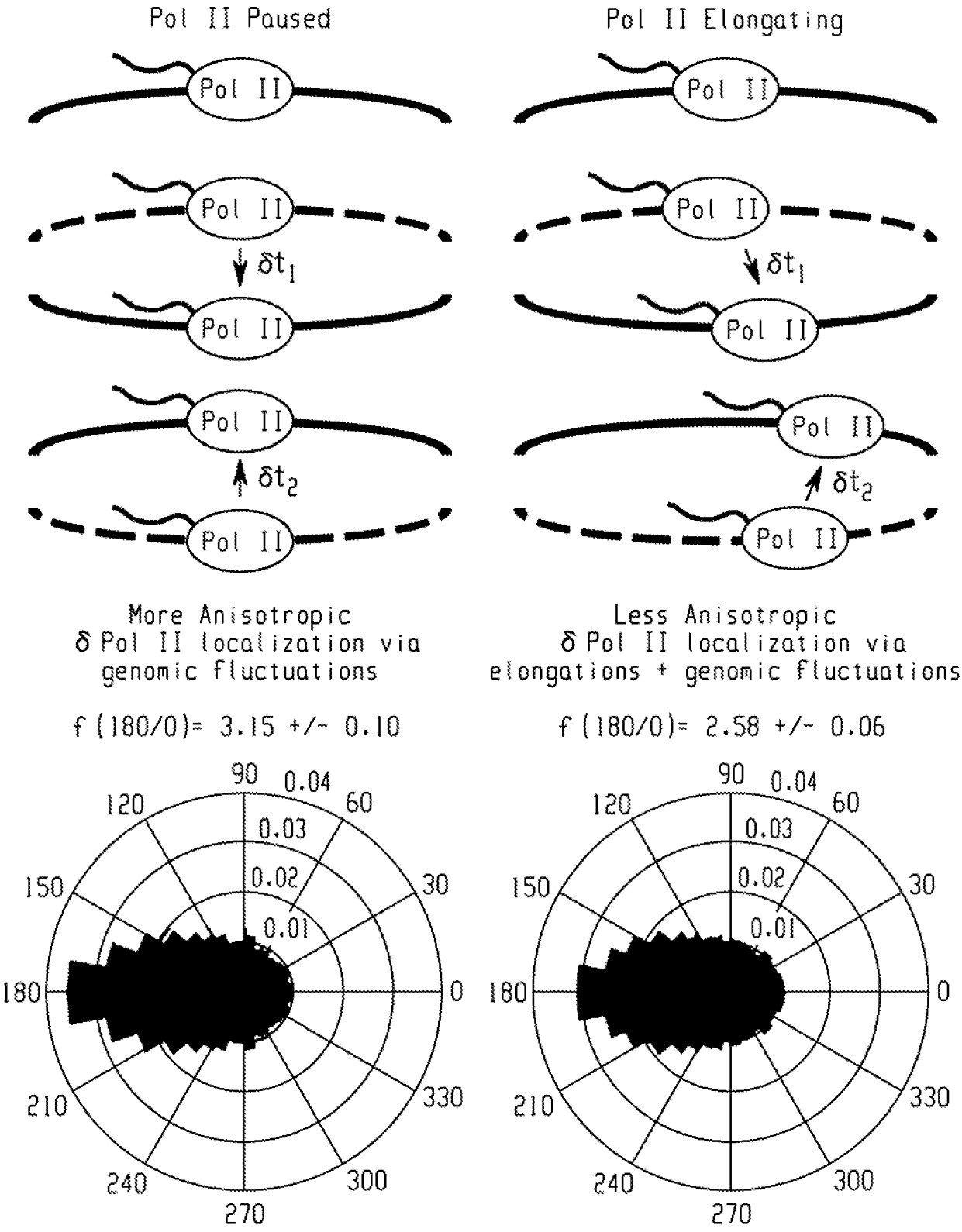

Pol II Paused
Pol II Elongating
Pol II
δt1
δt2
More Anisotropic
δ Pol II localization via
genomic fluctuations
f (180/0)= 3.15 +/- 0.10
Less Anisotropic
δ Pol II localization via
elongations + genomic fluctuations
f (180/0)= 2.58 +/- 0.06
90
120
60
150
30
180
0
210
330
240
300
270
0.04
0.03
0.02
0.01

Fig. 2C

FIG. 3

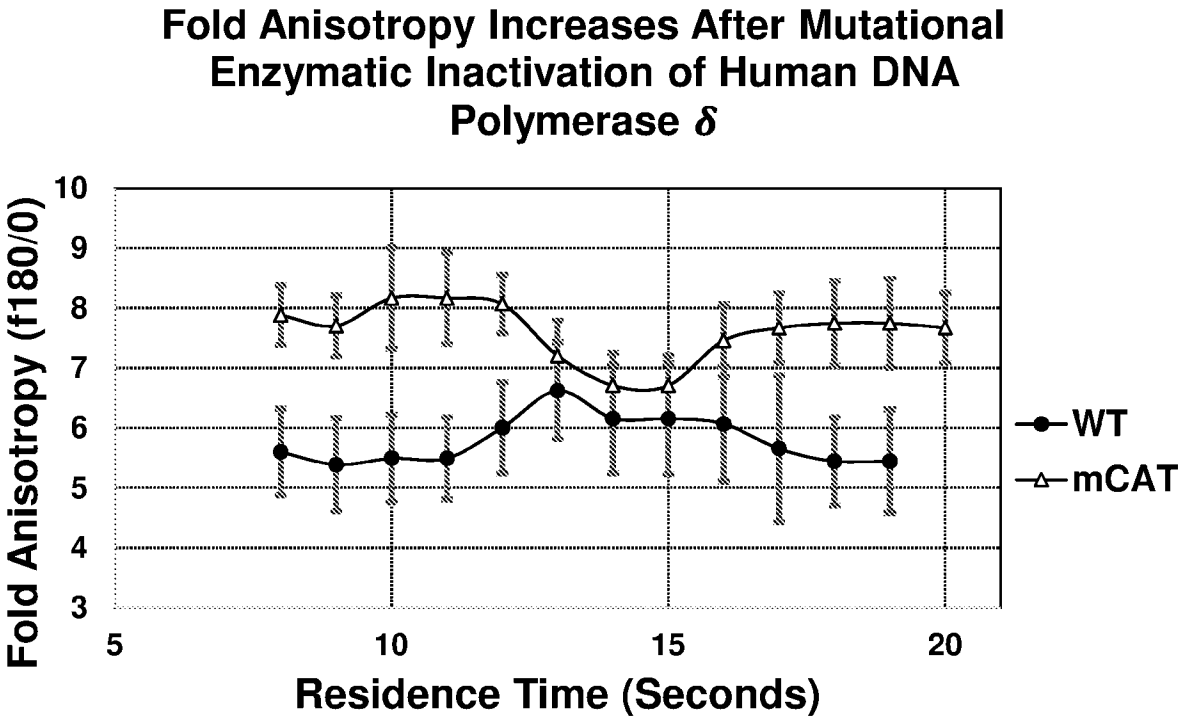

Fold Anisotropy Increases After Mutational Enzymatic Inactivation of Human DNA Polymerase δ
Fold Anisotropy (f180/0)
10
9
8
7
6
5
4
3
5
10
15
20
Residence Time (Seconds)
WT
mCAT

FIG. 4

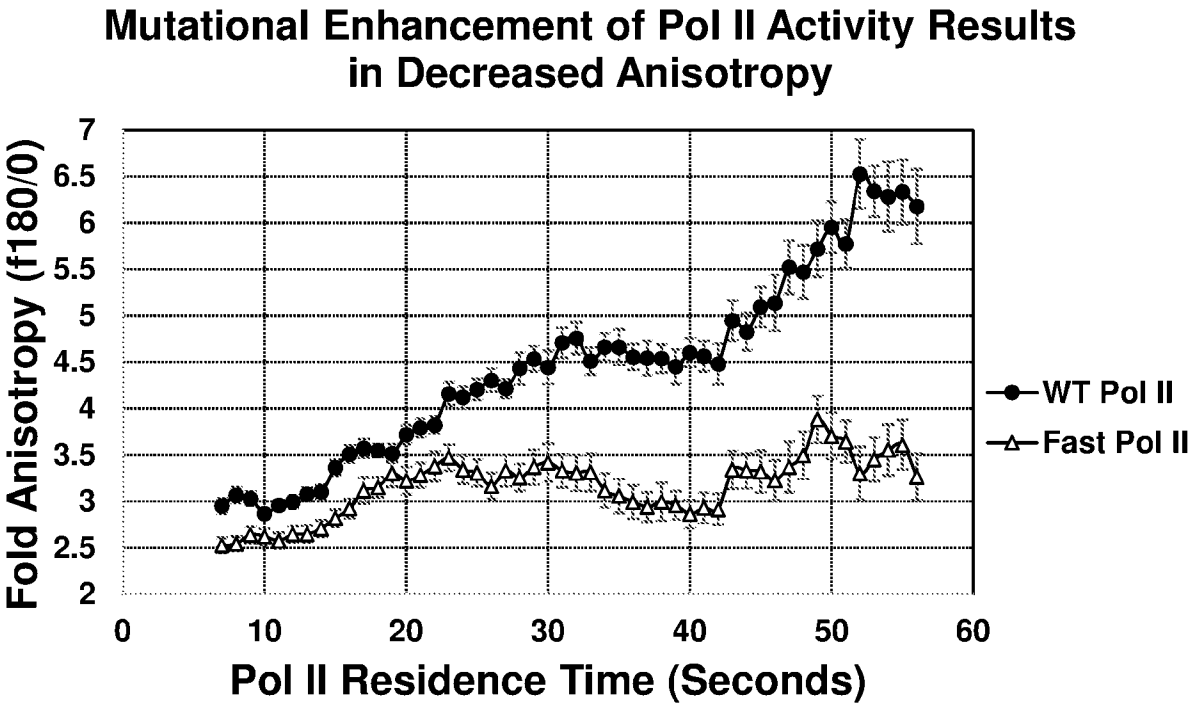

Mutational Enhancement of Pol II Activity Results in Decreased Anisotropy
Fold Anisotropy (f180/0)
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
0
10
20
30
40
50
60
Pol II Residence Time (Seconds)
WT Pol II
Fast Pol II

FIG. 5

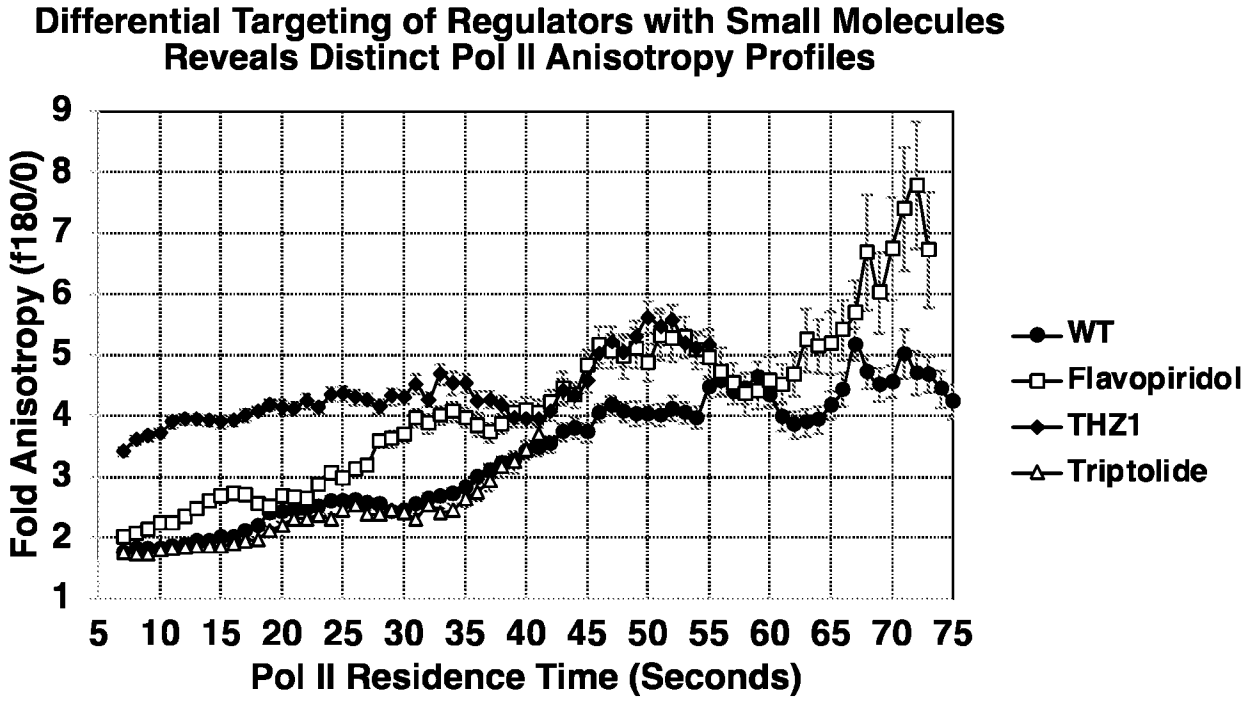
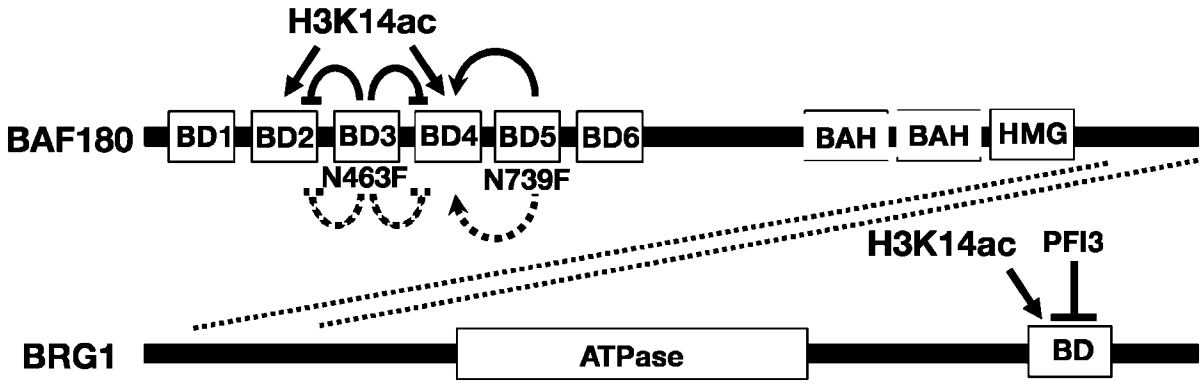

Differential Targeting of Regulators with Small Molecules
Reveals Distinct Pol II Anisotropy Profiles
Fold Anisotropy (f180/0)
9
8
7
6
5
4
3
2
1
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Pol II Residence Time (Seconds)
WT
Flavopiridol
THZ1
Triptolide

FIG. 6A


H3K14ac
BAF180
BD1
BD2
BD3
BD4
BD5
BD6
BAH
BAH
HMG
N463F
N739F
H3K14ac
PFI3
BRG1
ATPase
BD

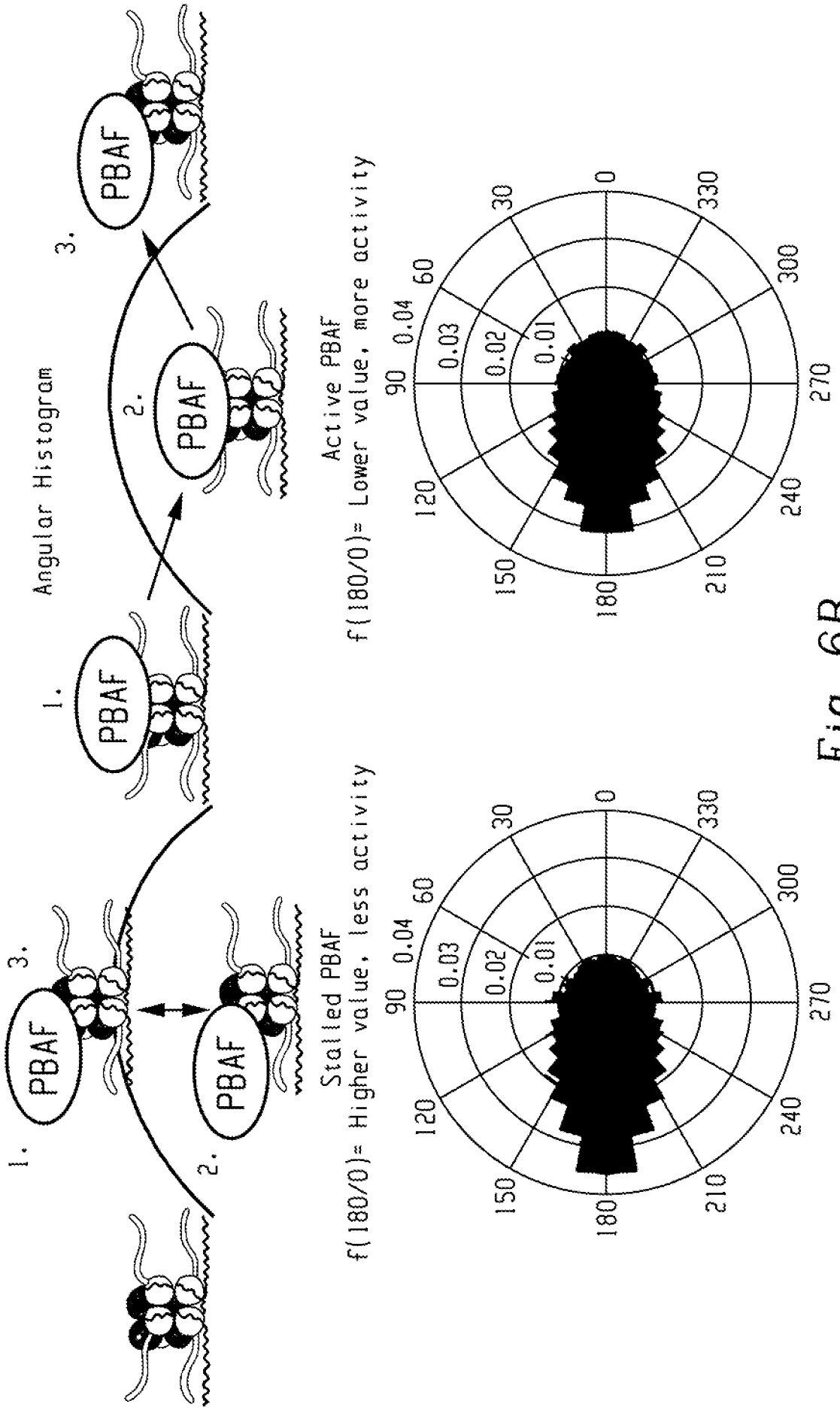

1.
PBAF
3.
2.
PBAF
Stalled PBAF
f(180/0)= Higher value, less activity
Angular Histogram
1.
PBAF
2.
PBAF
3.
PBAF
Active PBAF
f(180/0)= Lower value, more activity
0
30
60
90
120
150
180
210
240
270
300
330
0.01
0.02
0.03
0.04

Fig. 6B

FIG. 6C

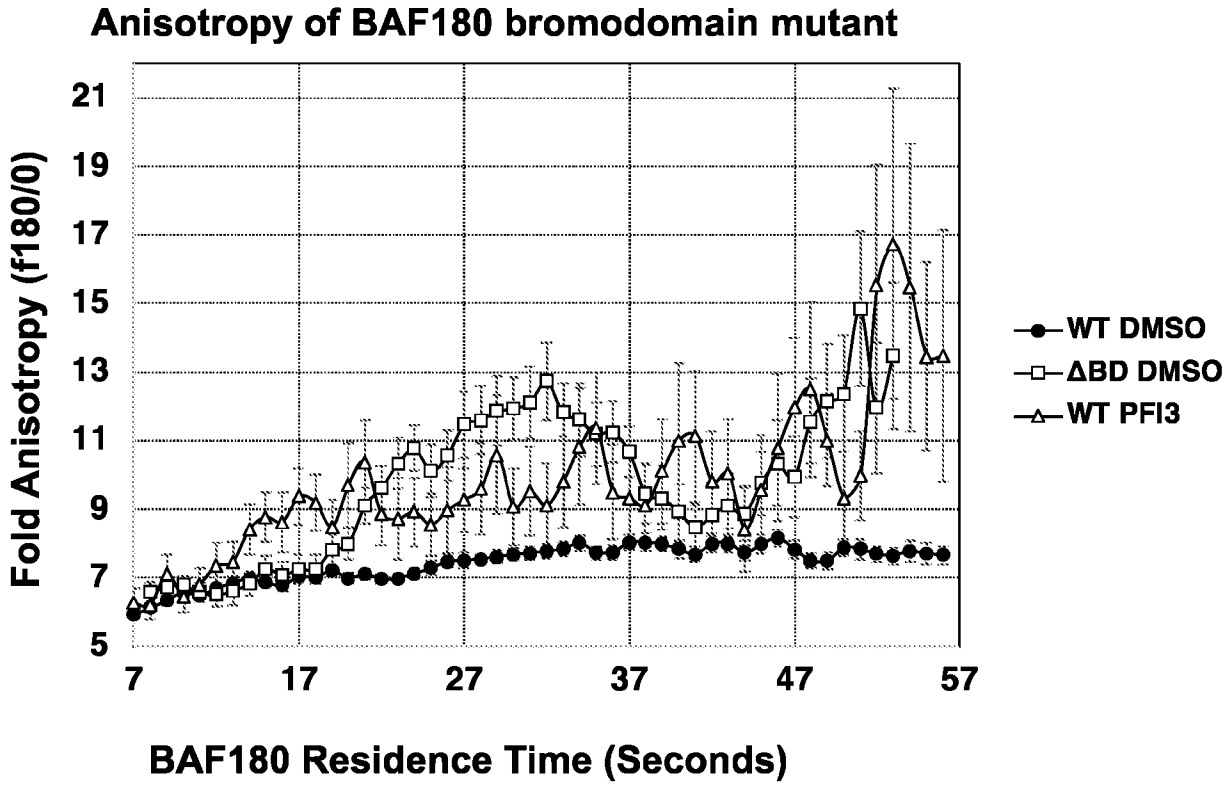

Anisotropy of BAF180 bromodomain mutant
Fold Anisotropy (f180/0)
21
19
17
15
13
11
9
7
5
7
17
27
37
47
57
BAF180 Residence Time (Seconds)
WT DMSO
ΔBD DMSO
WT PFI3

FIG. 6D

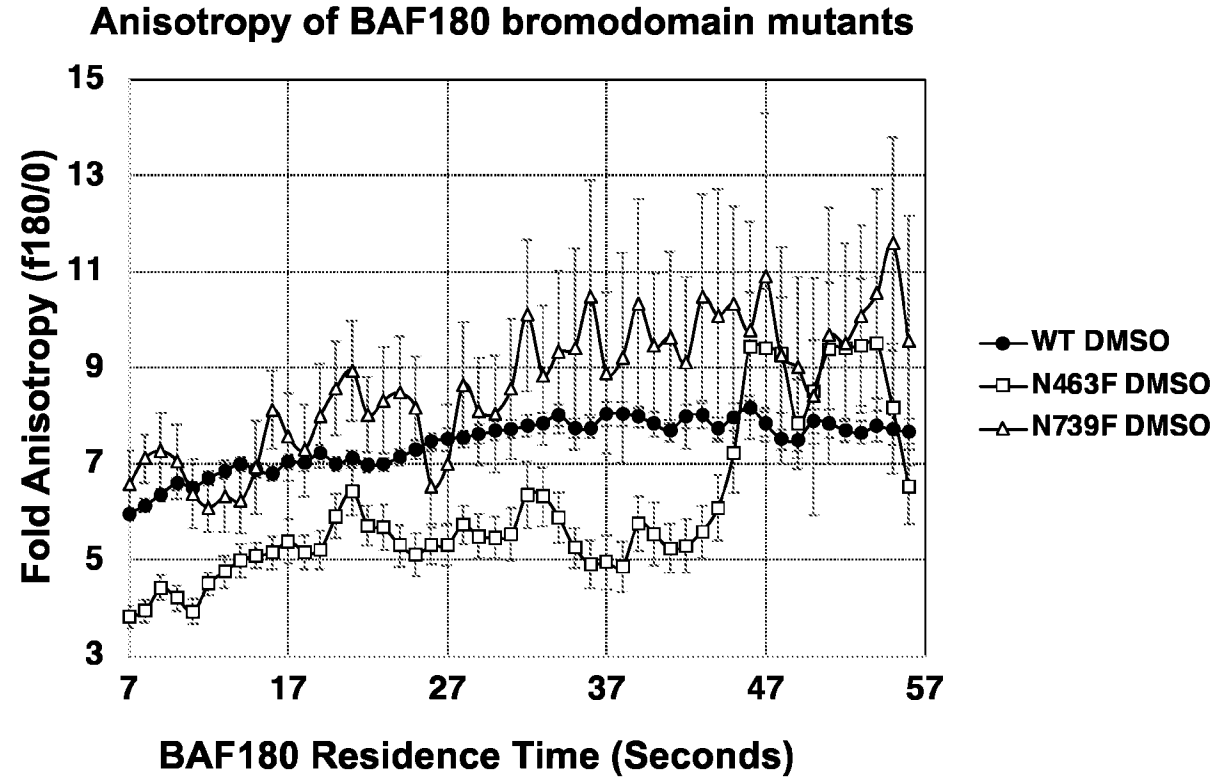

Anisotropy of BAF180 bromodomain mutants
Fold Anisotropy (f180/0)
15
13
11
9
7
5
3
7
17
27
37
47
57
BAF180 Residence Time (Seconds)
WT DMSO
N463F DMSO
N739F DMSO

FIG. 6E

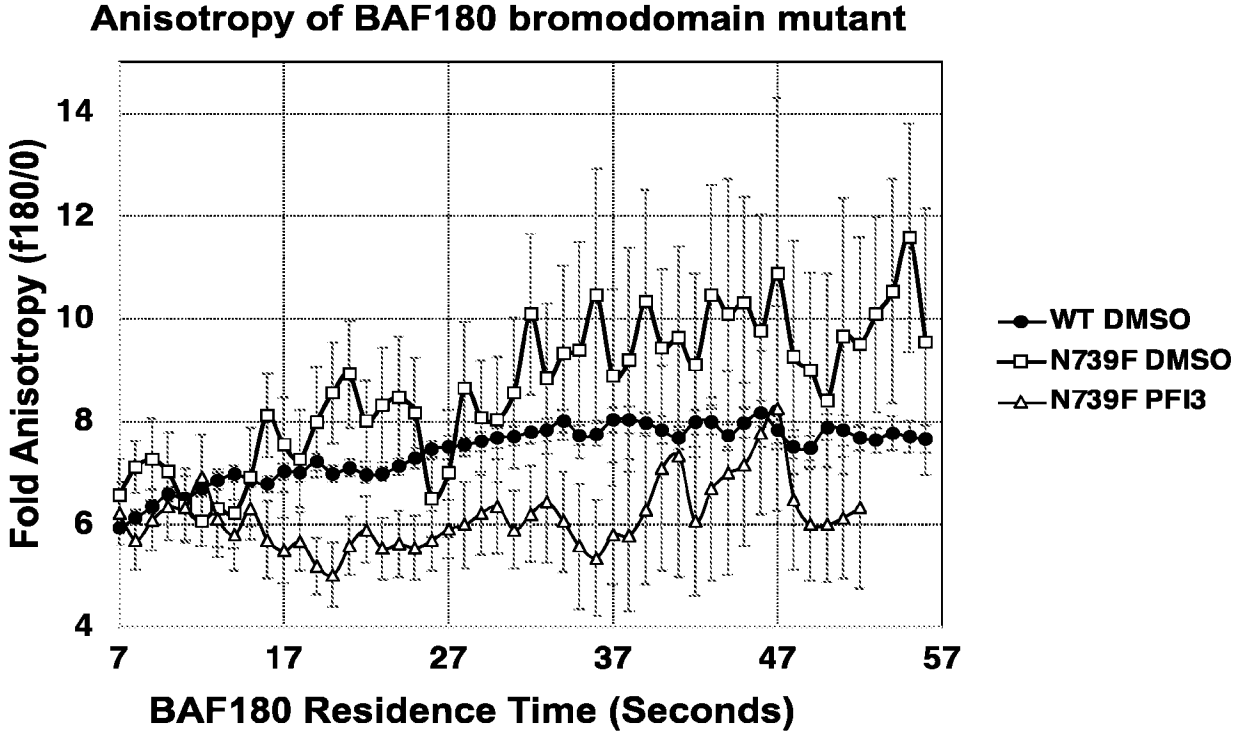

Anisotropy of BAF180 bromodomain mutant
Fold Anisotropy (f180/0)
14
12
10
8
6
4
7
17
27
37
47
57
BAF180 Residence Time (Seconds)
WT DMSO
N739F DMSO
N739F PFI3

FIG. 7

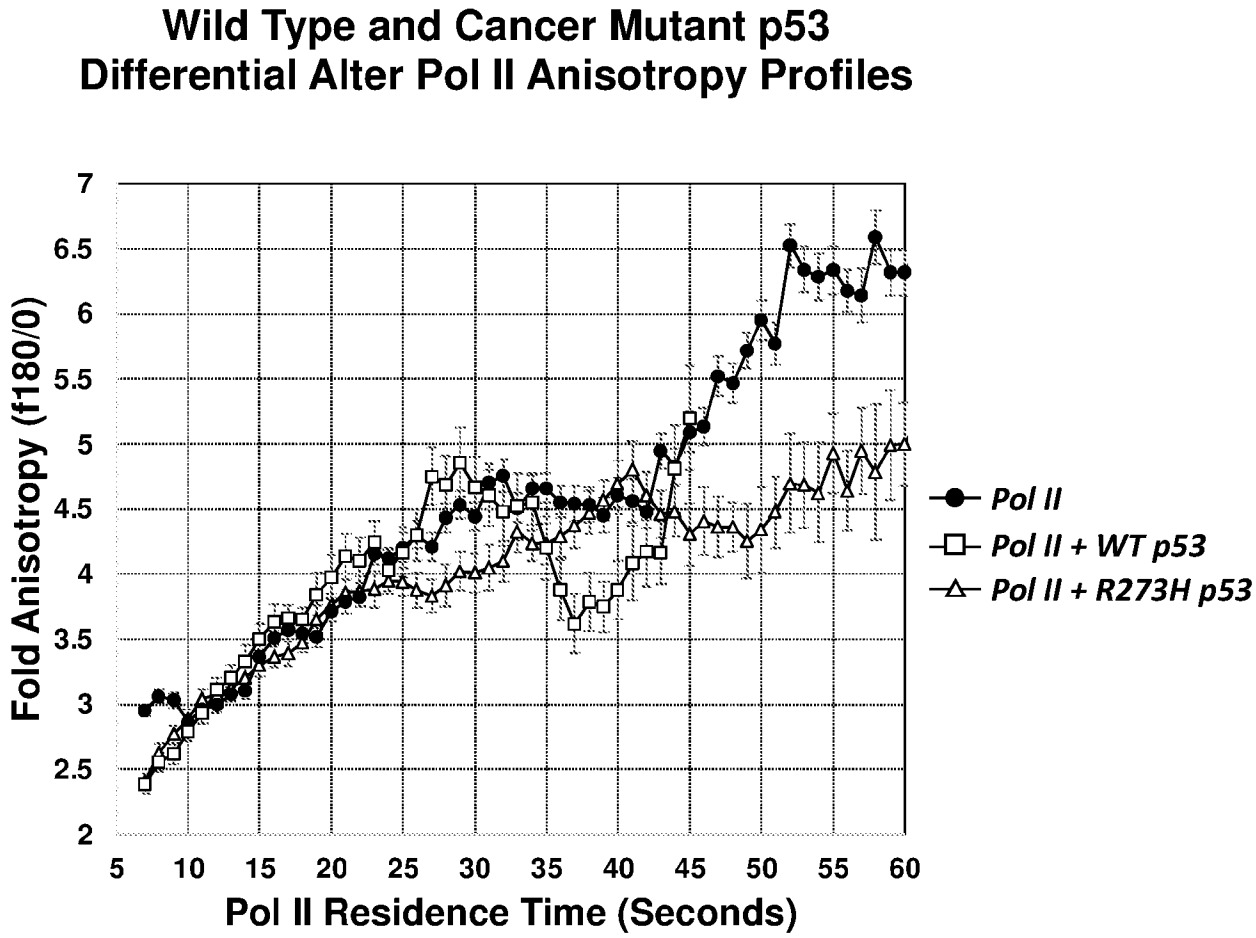

Wild Type and Cancer Mutant p53
Differential Alter Pol II Anisotropy Profiles
Fold Anisotropy (f180/0)
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
5
10
15
20
25
30
35
40
45
50
55
60
Pol II Residence Time (Seconds)
Pol II
Pol II + WT p53
Pol II + R273H p53

FIG. 8

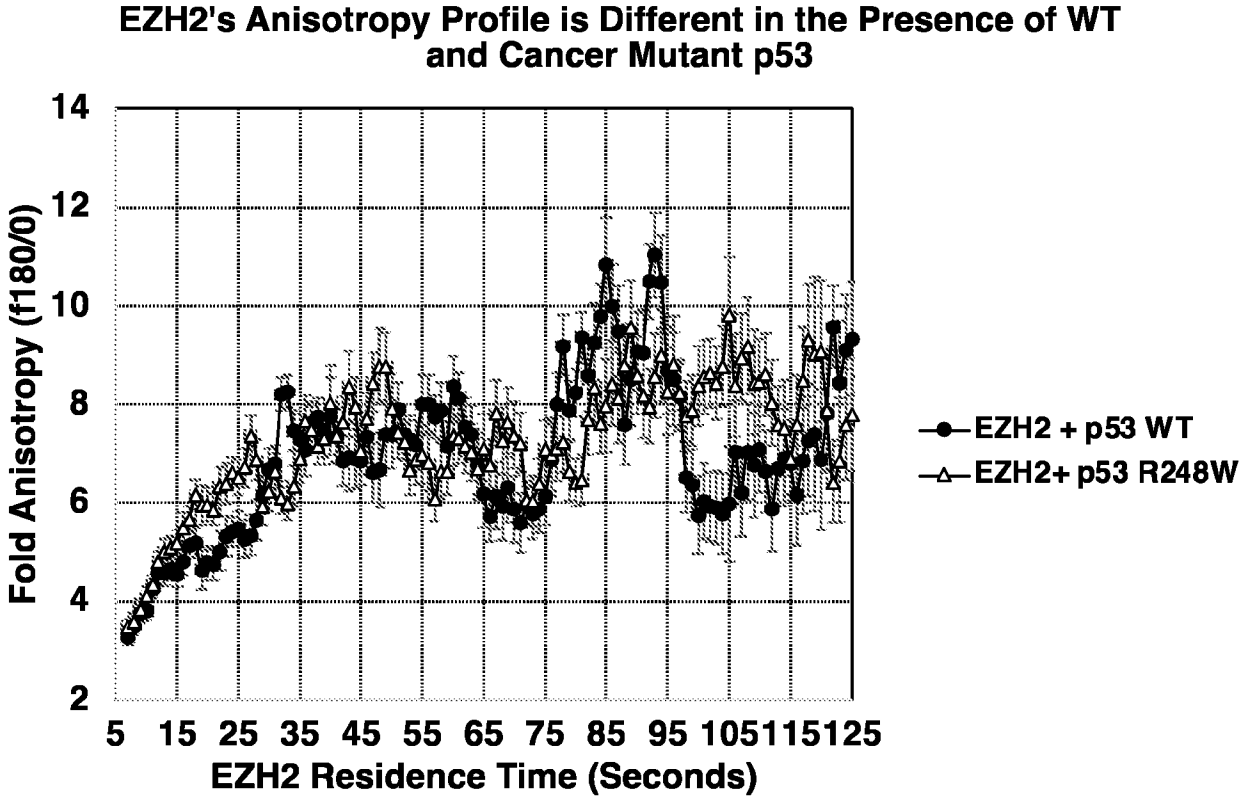

EZH2's Anisotropy Profile is Different in the Presence of WT and Cancer Mutant p53
Fold Anisotropy (f180/0)
14
12
10
8
6
4
2
5 15 25 35 45 55 65 75 85 95 105115125
EZH2 Residence Time (Seconds)
EZH2 + p53 WT
EZH2+ p53 R248W

FIG. 9A

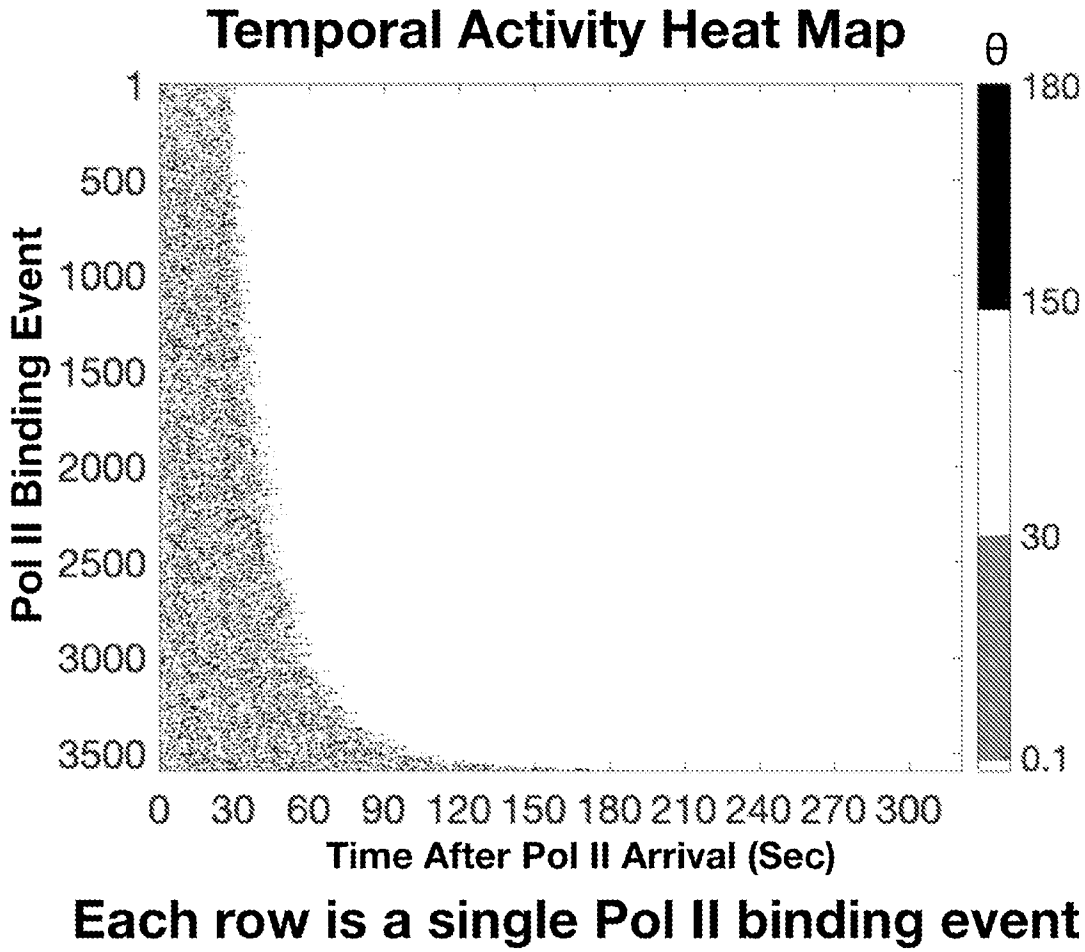

Temporal Activity Heat Map
θ
180
150
30
0.1
Pol II Binding Event
1
500
1000
1500
2000
2500
3000
3500
0 30 60 90 120 150 180 210 240 270 300
Time After Pol II Arrival (Sec)
Each row is a single Pol II binding event

FIG. 9B

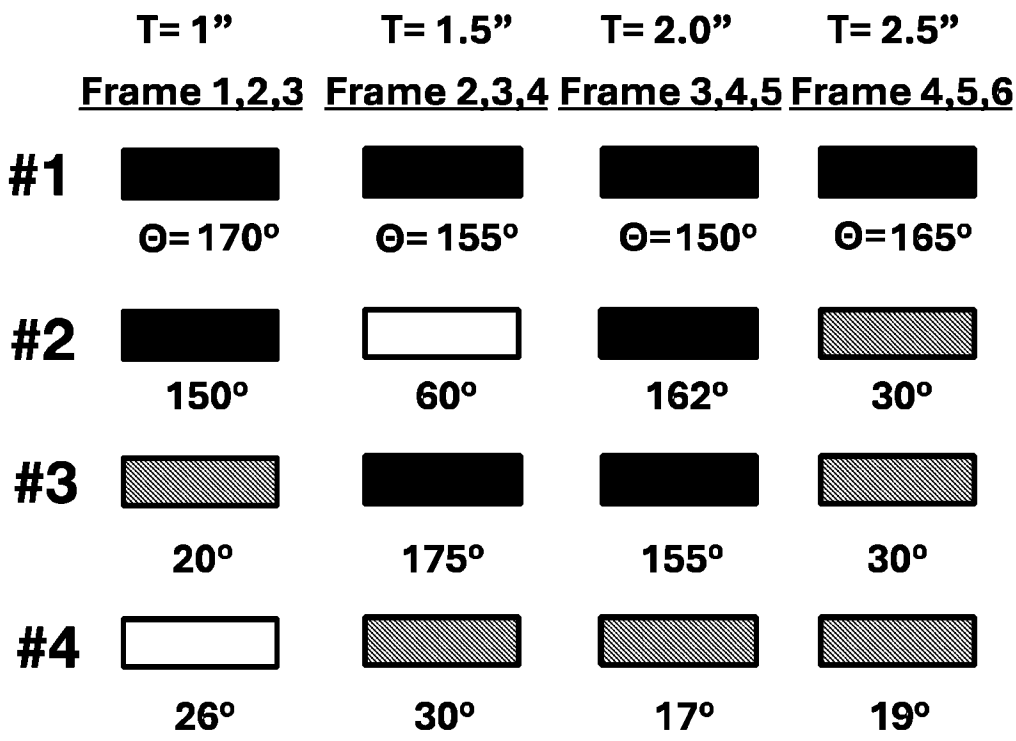

T= 1"
T= 1.5"
T= 2.0"
T= 2.5"
Frame 1,2,3
Frame 2,3,4
Frame 3,4,5
Frame 4,5,6
#1
Θ=170°
Θ=155°
Θ=150°
Θ=165°
#2
150°
60°
162°
30°
#3
20°
175°
155°
30°
#4
26°
30°
17°
19°

FIG. 9C

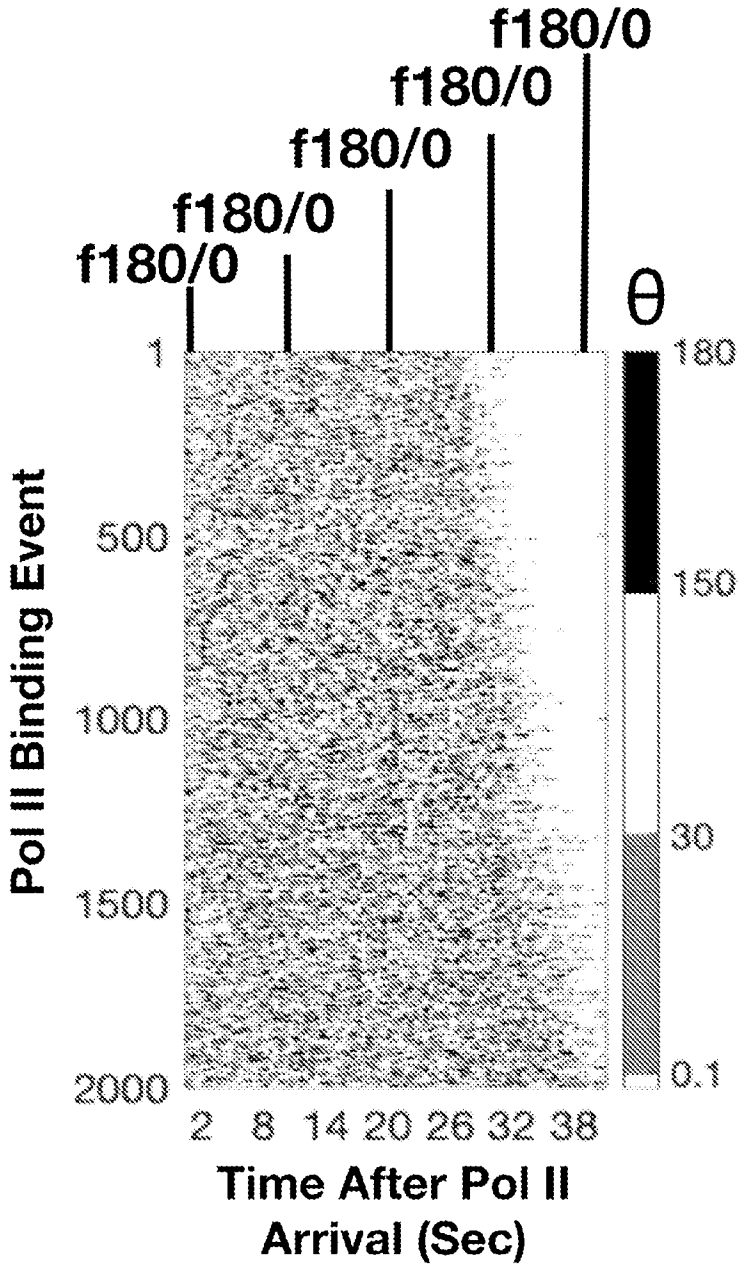

f180/0
f180/0
f180/0
f180/0
f180/0
θ
180
150
30
0.1
1
500
1000
1500
2000
Pol II Binding Event
2 8 14 20 26 32 38
Time After Pol II Arrival (Sec)

FIG. 9D

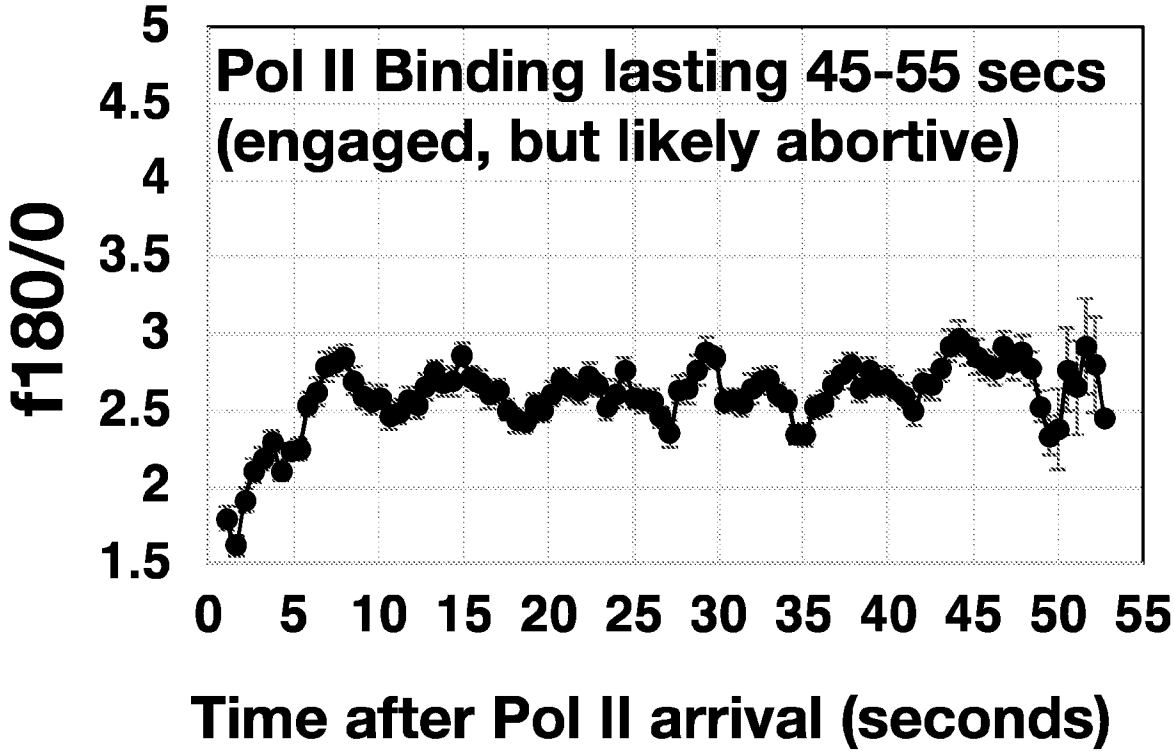
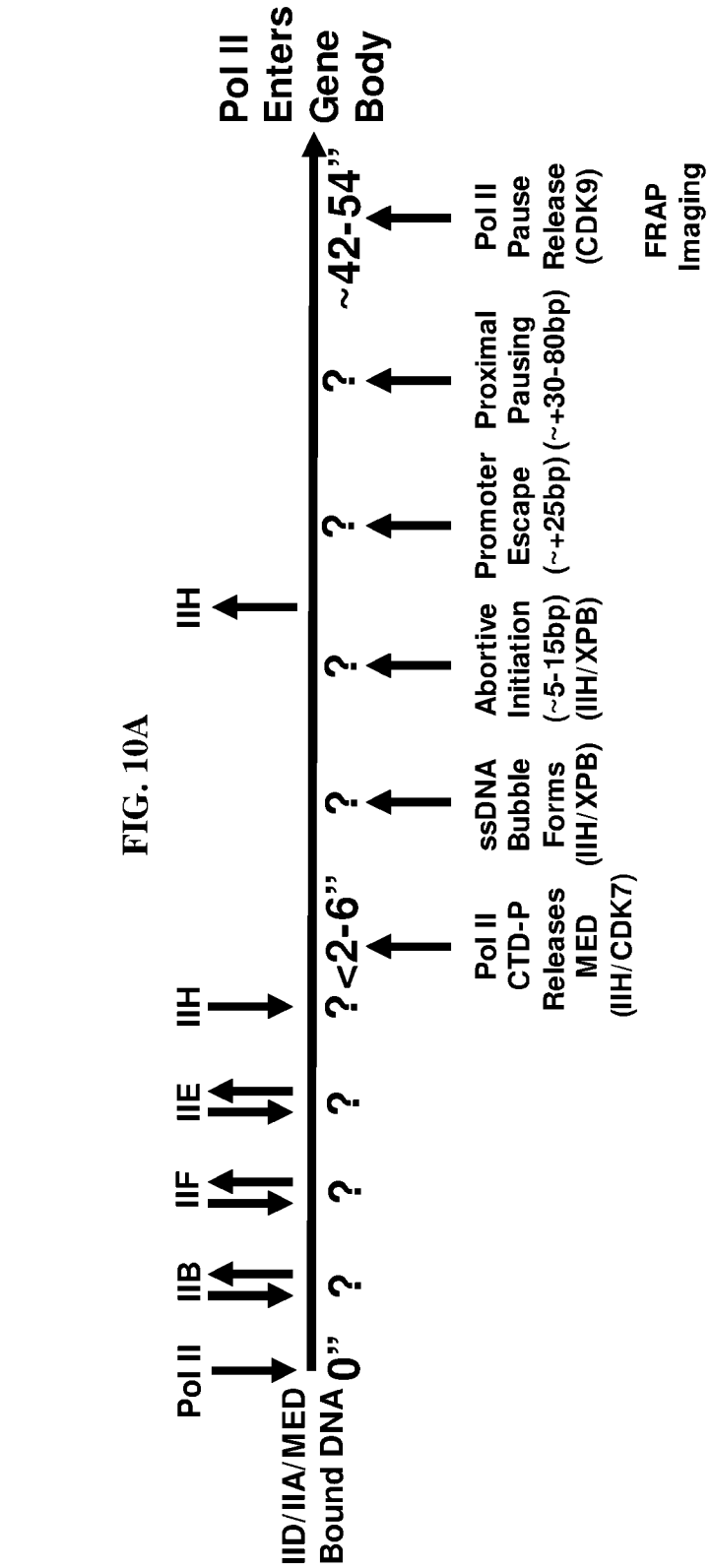

Pol II Binding lasting 45-55 secs
(engaged, but likely abortive)
f180/0
5
4.5
4
3.5
3
2.5
2
1.5
0 5 10 15 20 25 30 35 40 45 50 55
Time after Pol II arrival (seconds)

FIG. 10A


IID/IIA/MED
Bound DNA
Pol II
IIB
IIF
IIE
IIH
0”
?
?
?
?<2-6”
?
?
?
~42-54”
Pol II Enters Gene Body
Pol II CTD-P Releases MED (IIH/CDK7)
ssDNA Bubble Forms (IIH/XPB)
Abortive Initiation (~5-15bp) (IIH/XPB)
Promoter Escape (~+25bp)
Proximal Pausing (~+30-80bp)
Pol II Pause Release (CDK9)
FRAP Imaging

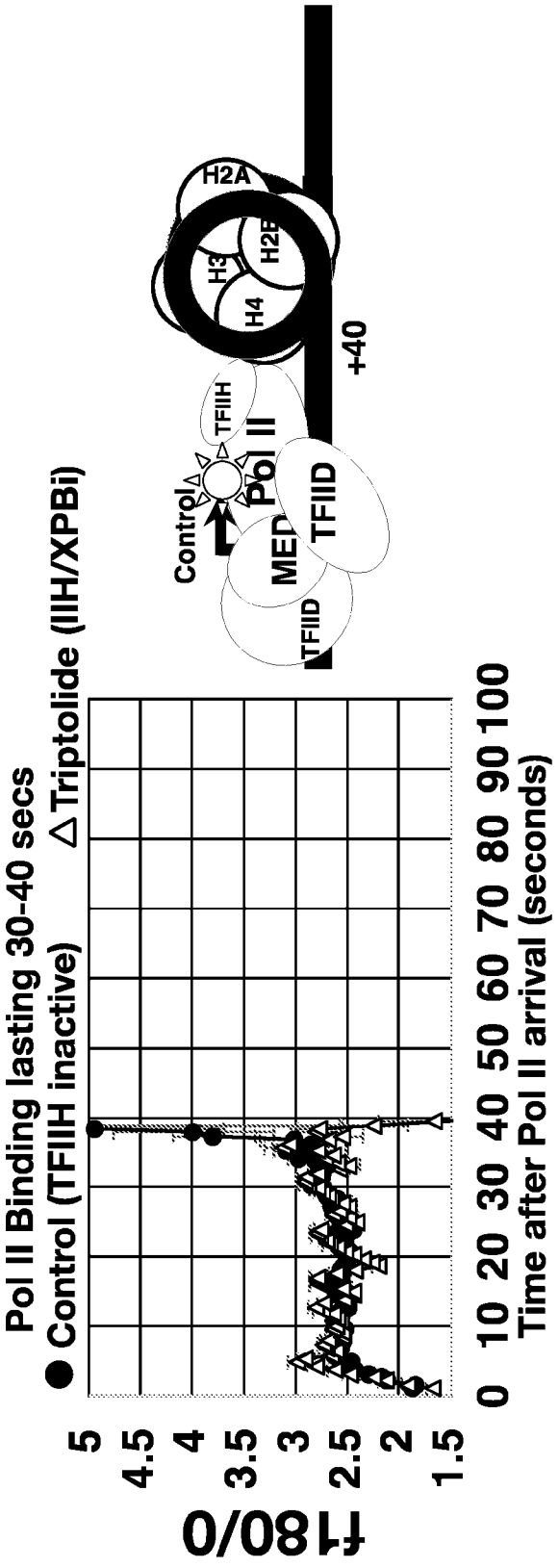

f180/0
5
4.5
4
3.5
3
2.5
2
1.5
0 10 20 30 40 50 60 70 80 90 100
Time after Pol II arrival (seconds)
Pol II Binding lasting 30-40 secs
● Control (TFIIH inactive)
△ Triptolide (IIH/XPBi)
Control
TFIIH
Pol II
MED
TFIID
TFIID
H2A
H2B
H3
H4
+40

FIG. 10B

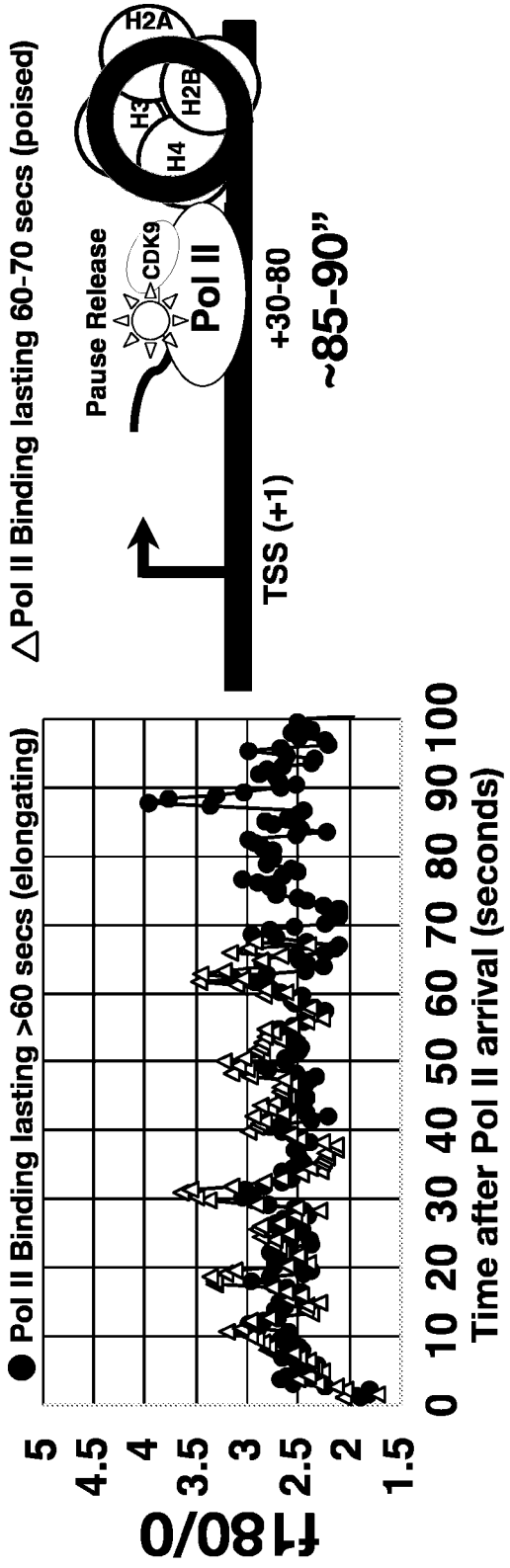

f180/0
5
4.5
4
3.5
3
2.5
2
1.5
0 10 20 30 40 50 60 70 80 90 100
Time after Pol II arrival (seconds)
● Pol II Binding lasting >60 secs (elongating)
△Pol II Binding lasting 60-70 secs (poised)
TSS (+1)
+30-80
~85-90”
Pause Release
CDK9
Pol II
H2A
H2B
H3
H4

FIG. 10C

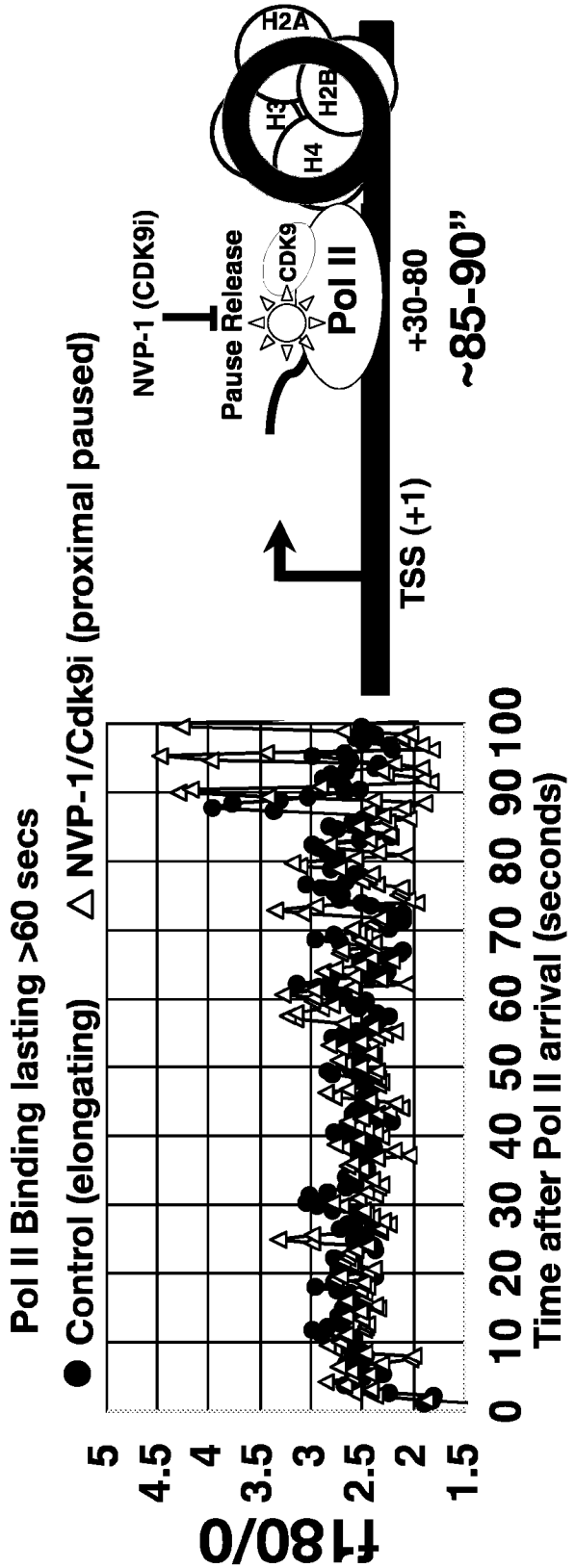

H2A
H2B
H3
H4
CDK9
Pol II
NVP-1 (CDK9i)
Pause Release
+30-80
~85-90”
TSS (+1)
Pol II Binding lasting >60 secs
● Control (elongating) △ NVP-1/Cdk9i (proximal paused)
f180/0
5
4.5
4
3.5
3
2.5
2
1.5
0 10 20 30 40 50 60 70 80 90 100
Time after Pol II arrival (seconds)

FIG. 10D

FIG. 11A

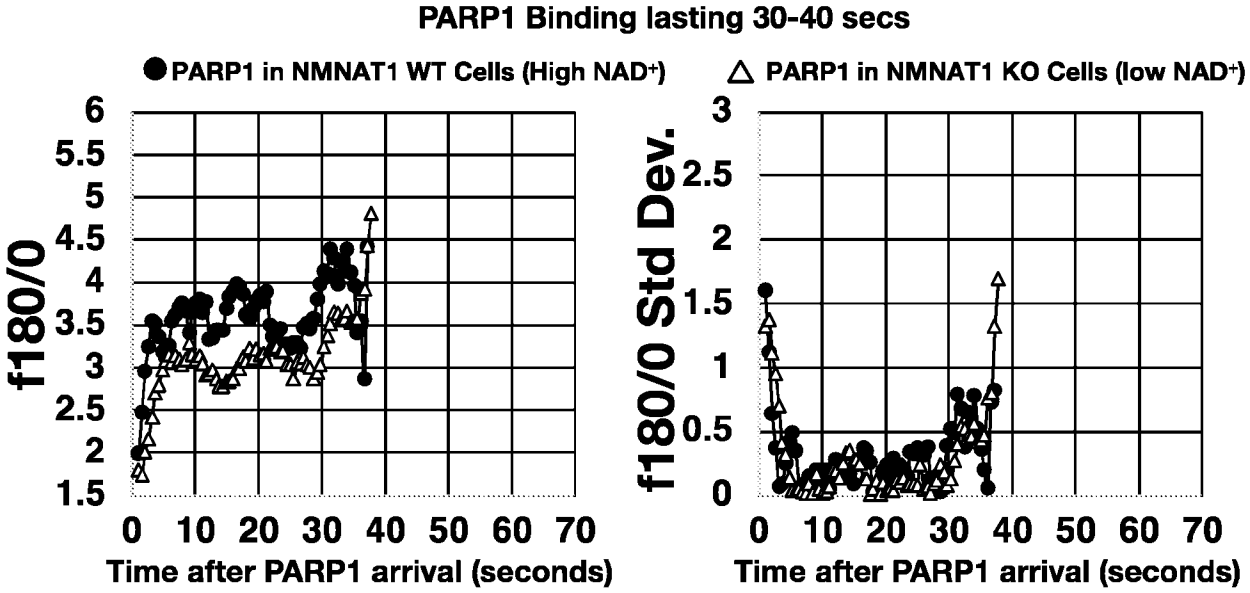


FIG. 11B