Nucleosome Remodeling Assay by EpiDyne™-FRET

Fully recombinant nucleosome substrates engineered for chromatin remodeling studies

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Overview

Chromatin remodeling, or the repositioning of nucleosomes, regulates DNA access and thus gene expression and genome repair. Many ATP-dependent remodeling enzyme complexes are associated with human disease but are challenging study targets due to the requirement for nucleosome-based substrates. EpiCypher has addressed this need by developing the EpiDyne platform of fully recombinant remodeling substrates to monitor nucleosome repositioning along DNA. Here, we demonstrate the utility of EpiDyne-FRET in a homogeneous remodeling assay using **F**luorescence **R**esonance **E**nergy **T**ransfer (**FRET**) readout.

Chromatin Remodeling Enzymes As Therapeutic Targets

Aberrant nucleosome organization can severely disrupt gene expression, DNA repair and cellular differentiation, and it also plays a major role in human disorders, including cancer, inflammation, autoimmunity, schizophrenia, cardiovascular disease, and intellectual disability. Remarkably, nearly 20% of all cancers contain mutations in subunits from the SWI/SNF family of ATP-dependent chromatin remodeling complexes. These enzyme complexes regulate local genome access by 'pumping' the DNA around histone octamers, thus 'sliding' nucleosomes [1]. Recurrent somatic mutations in SWI/SNF subunits are observed in multiple cancers, supporting a driver role in tumorigenesis [2]. The mutated remodeling proteins are attractive therapeutic targets, since further compromising their ATPase activity promotes cancer cell death but spares normal cells [2, 3]. This phenomenon is known as synthetic lethality, and identifying inhibitors to exploit it may lead to drugs with cancer specificity [4, 5].

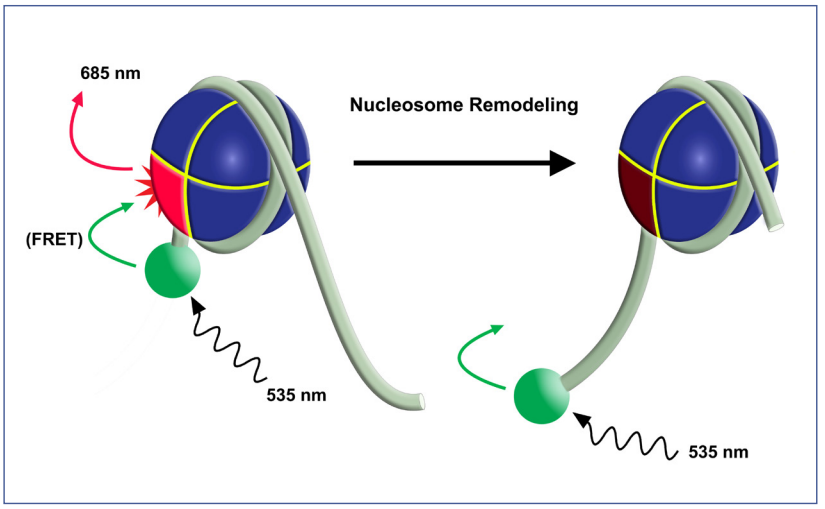

Figure 1: EpiDyne-FRET Nucleosome Remodeling Substrates consist of a Cy5-labeled human histone octamer (H2A T120C-Cy5; shown as red section of octamer) wrapped by 5' Cy3-labeled DNA (217bp; green ball) comprising a terminally nucleosome positioning sequence (147bp Widom 601 [7]) adjacent to a TGGA-repeat region refractory to nucleosome assembly [8]. In its assembled starting state, Cy3-Cy5 FRET is at a maximum. The activity of an ATP-dependent remodeler (*e.g.* RSC or another SWI/SNF ATPase) is detected by a reduction in FRET signal as the Cy3-labeled DNA 5' end is moved away from the Cy5-labeled octamer. EpiDyne-FRET is a one-step no-wash method immediately compatible with HTS applications.

Chromatin Remodeling Assays Using Recombinant Mononucleosome Substrates

EpiCypher has developed EpiDyne-FRET to study the function of chromatin remodeling enzyme complexes. The nucleosome consists of a core histone octamer wrapped in ~147 bp DNA and represents the basic repeating unit of chromatin. EpiDyne-FRET is comprised of a terminally positioned histone octamer (H2A-T120C*Cy5) wrapped in 5' Cy3-labelled DNA [8] (**Figure 1**). In its assembled start position, Cy3-Cy5 FRET is at maximum, with nucleosome remodeling detected by loss of the Cy5 signal as the histone octamer is relocated (towards the template DNA 3' end) or ejected.

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Nucleosome Remodeling Assay by Cy3/Cy5 EpiDyne™-FRET

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Reagents and Materials Required for the Assay [see Note (i)]

- Yeast RSC [9] (substrate also compatible with dACF [ACF-ISWI], dNoRC [Toutatis-ISWI] and human SMARCA2/4 (BRG1/BRM); data not shown)
- EpiDyne-FRET nucleosome remodeling substrate (EpiCypher Catalog No. 16-4201)
- Assay Buffer (20 mM Tris pH 7.5, 50 mM KCl, 3 mM $MgCl_2$ and 0.1 mg/mL BSA (Sigma Catalog No. A3059)
- ATP (Invitrogen Catalog No. PV3227)
- Optional: ATPγS (a non-hydrolyzable form of ATP)
- Corning 3820 384-well assay plate (Fisher Catalog No. 07-200-891)
- 16 channel multipipetter for 384-well plates
- 384-well Fluorescence microplate reader capable of Cy3/Cy5-FRET detection (*e.g.* Envision, Perkin Elmer)

Standard Protocol

1. Determine the amount of RSC (10 nM final), EpiDyne-FRET (20 nM final), and ATP (2 mM final) needed - based on the intended time points / replicates (triplicate shown) [see Notes (ii & iii)].
2. Prepare reaction components (room temp).
   i. **2x Enzyme/Substrate solution** (20 nM RSC + 40 nM EpiDyne-FRET substrate in **2x Assay Buffer**)
   ii. **2x ATP +/- inhibitor solution(s)** (4 mM ATP in ddH_2O + inhibitors [*e.g.* ATPγS] as required)
3. Add 5 µL of **2x Enzyme/Substrate solution** to the microplate.
4. Initiate reactions by adding 5 µL of the **2x ATP +/- inhibitor solution**.
5. At appropriate timepoints read in a 384-well plate reader capable of Cy3 (excitation-531 nm / emission-579 nm) / Cy5 (emission-685 nm) detection.
6. Data is expressed as the ratio of the raw Cy3 and Cy5 emission signals at each time point [8].

NOTES

(i) Store protein aliquots at -80°C and avoid freeze/thaw.

(ii) For assay development it is advised to titrate the concentration of multiple reaction components, including enzyme, substrate, and ATP.

(iii) Since data is expressed as Cy3-Cy5 emission it is important not to add excess substrate to these reactions.

(iv) Reactions can begin rapidly. If setting up a large plate without an auto-injector there is no capability of a T0 for each condition.

References

1. Hamiche, A., et al., ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. Cell, 1999. 97(7): p. 833-42.
2. Vangamudi, B., et al., The SMARCA2/4 ATPase Domain Surpasses the Bromodomain as a Drug Target in SWI/SNF-Mutant Cancers: Insights from cDNA Rescue and PFI-3 Inhibitor Studies. Cancer Res, 2015. 75(18): p. 3865-78.
3. Wu, Q., et al., Targeting the chromatin remodeling enzyme BRG1 increases the efficacy of chemotherapy drugs in breast cancer cells. Oncotarget, 2016. 7(19): p. 27158-75.
4. Helming, K.C., X. Wang, and C.W. Roberts, Vulnerabilities of mutant SWI/SNF complexes in cancer. Cancer Cell, 2014. 26(3): p. 309-17.
5. Karnezis, A.N., et al., Dual loss of the SWI/SNF complex ATPases SMARCA4/BRG1 and SMARCA2/BRM is highly sensitive and specific for small cell carcinoma of the ovary, hypercalcaemic type. J Pathol, 2016. 238(3): p. 389-400.
6. Clapier, C.R., et al., Regulation of DNA Translocation Efficiency within the Chromatin Remodeler RSC/Sth1 Potentiates Nucleosome Sliding and Ejection. Mol Cell, 2016. 62(3): p. 453-61.
7. Lowary, P.T. and J. Widom, New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. J Mol Biol, 1998. 276(1): p. 19-42.
8. Cao, H., et al., TGGA repeats impair nucleosome formation. J Mol Biol, 1998. 281(2): p. 253-60.

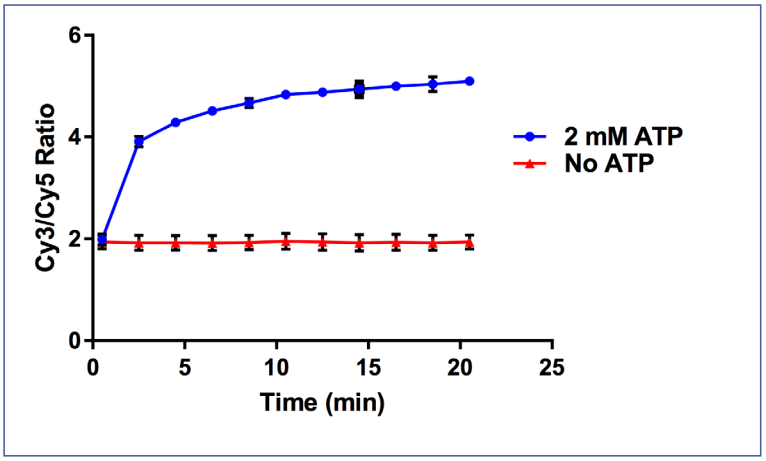

Figure 2: RSC/ATP-dependent nucleosome remodeling reaction. EpiDyne-FRET nucleosomes (20 nM) were incubated with RSC chromatin remodeler (10 nM) in the presence or absence of 2 mM ATP (conditions previously determined as optimal for this enzyme: data not shown). Upon ATP addition, reactions were read in an Envision Multi-label plate reader at times indicated. Data is presented as the mean±SD of the Cy3/Cy5 ratio (N=6) [see Note (iv)].

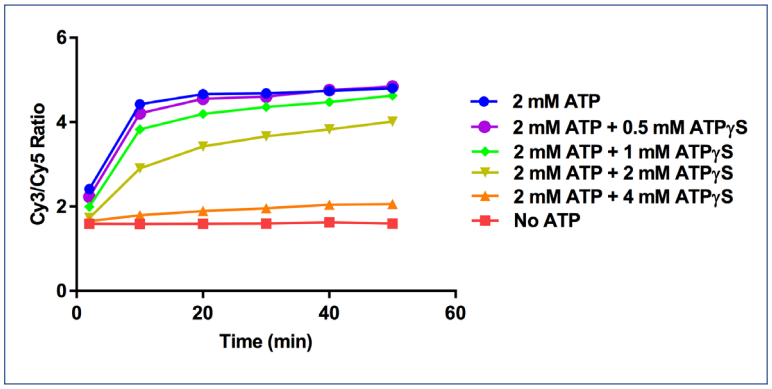

Figure 3: ATPγS titration with fixed ATP. EpiDyne-FRET nucleosomes (20 nM) were incubated with RSC (10 nM) in the presence of fixed 2 mM ATP with increasing amounts of ATPγS. Upon ATP addition, reactions were immediately read in an Envision Multi-label plate reader. Data is presented as the mean of the Cy3-Cy5 ratio (N=2) [see Note (iv)].

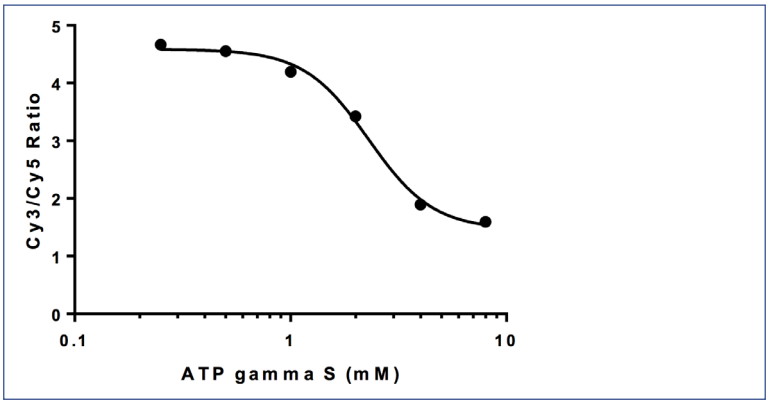

Figure 4: IC_{50} calculation for ATPγS. Data from Figure 3 (20-minute time point) were analyzed to determine the IC_{50} value for ATPγS (2.29 mM). Data is presented as the mean of the Cy3-Cy5 ratio vs. the ATPγS concentration.

Acknowledgments

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