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Evaluating drug-chromatin interactions using novel methods for target engagement studies

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Abstract

Regulation of gene expression is a dynamic process orchestrated and maintained by a large variety of chromatin-interacting proteins. Understanding these chromatin-protein complexes and their binding kinetics is crucial for development of the small-molecule drugs. Promega has developed NanoBRET®? technology, which allows for dynamic measurement of protein:protein interactions (PPIs) in living cells. This proximity-based assay measures energy transfer from a bioluminescent protein donor (NanoLuc® fusion protein) to a fluorescent protein acceptor (HaloTag® fusion protein) under physiological conditions. Furthermore, the assessment of chromatin-protein interaction reversibility is also possible allowing for identification of compounds that either induce or inhibit the target complex. Evaluating the engagement of these small molecules with chromatin regulatory proteins provides useful information for chemical probe optimization and further pharmaceutical development. In addition to the specificity and affinity of target engagement, binding dynamics under non-equilibrium conditions may also underlie the therapeutic potential of the drugs. Promega has developed NanoBRET®? Target Engagement system, wherein the apparent binding affinity and permeability of test compound is measured by competitive displacement of a broad-coverage NanoBRET®? Tracer reversibly bound to a NanoLuc fusion protein in cells.

As both the compound and tracer compete directly for the same binding site it enables quantification of drug residence time on the targets. During this talk the author will discuss the work they have completed using histone deacetylases (HDACs) and bromodomain (BRD)-containing proteins as the targets in our NanoBRET PPI and target engagement studies.

Malfunctions in the basic epigenetic mechanisms such as histone modifications, DNA methylation, and chromatin remodeling are implicated in a number of cancers and immunological and neurodegenerative conditions. Within GlaxoSmithKline (GSK) we have utilized a number of variations of the NanoBRET technology for the direct measurement of compound–target engagement within native cellular environments to drive high-throughput, routine structure–activity relationship (SAR) profiling across differing epigenetic targets. NanoBRET is a variation of the bioluminescence resonance energy transfer (BRET) methodology utilizing proteins of interest fused to either NanoLuc, a small, high-emission-intensity luciferase, or HaloTag, a modified dehalogenase enzyme that can be selectively labeled with a fluorophore. The combination of these two technologies has enabled the application of NanoBRET to biological systems such as epigenetic protein–protein interactions, which have previously been challenging. By synergizing target engagement assays with more complex primary cell phenotypic assays, we have been able to demonstrate compound–target selectivity profiles to enhance cellular potency and offset potential liability risks. Additionally, we have shown that in the absence of a robust, cell phenotypic assay, it is possible to utilize NanoBRET target engagement assays to aid chemistry in progressing at a higher scale than would have otherwise been achievable. The NanoBRET target engagement assays utilized have further shown an excellent correlation with more reductionist biochemical and biophysical assay systems, clearly demonstrating the possibility of using such assay systems at scale, in tandem with, or in preference to, lower-throughput cell phenotypic approaches.

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