Technology Offer

High-Resolution Protein–Nucleic Acid Interaction Mapping with Engineered N6-Methyltransferase

Ref.-No.: 0707-6914-BC

Abstract

This invention introduces a genetically engineered, sequence-unspecific N6-methyltransferase capable of covalently labeling both DNA and RNA in close proximity to a protein-of-interest (POI). By introducing targeted amino acid substitutions at specific positions in the enzyme (e.g., W46, E44, C285, R336), the modified methyltransferase preferentially accepts artificial S-Adenosylmethionine (SAM) analogues carrying functional groups such as fluorescent dyes, affinity tags, or reactive moieties. The resulting covalent labels are stable through nucleic acid isolation and enable direct visualization or sequencing-based detection of POI proximity to nucleic acids. This method overcomes limitations of existing approaches (e.g., DamID, MadID, mTAG) by achieving higher labeling density, discriminating against natural SAM, and enabling simultaneous DNA and RNA mapping. The technology is compatible with fluorescence microscopy, super-resolution imaging, optical genome mapping, and direct nucleic acid modification detection via sequencing platforms such as Oxford Nanopore or PacBio. It offers a robust, versatile, and precise tool for studying dynamic protein–nucleic acid interactions at single-cell resolution.

Background

Understanding how proteins interact with nucleic acids is essential for unraveling gene regulation, chromatin architecture, RNA metabolism, and other cellular processes. Existing methods, such as chromosome conformation capture (3C/Hi-C) or DamID, have been valuable but present significant limitations. They often require complex workflows, large cell populations, or sequence-specific recognition sites, and they cannot easily detect RNA interactions. DamID and MadID approaches rely on natural methyltransferase cofactors, resulting in limited label density and instability of non-covalent tags during nucleic acid purification. Moreover, distinguishing experimental methyl marks from naturally occurring modifications, especially in RNA, remains challenging. The mTAG method allows covalent labeling but is restricted to sequence-specific enzymes and DNA targets. These constraints leave a clear need for a universal, high-resolution, covalent labeling method that works for both DNA and RNA, supports live or fixed-cell imaging, and integrates seamlessly with downstream sequencing or mapping technologies.

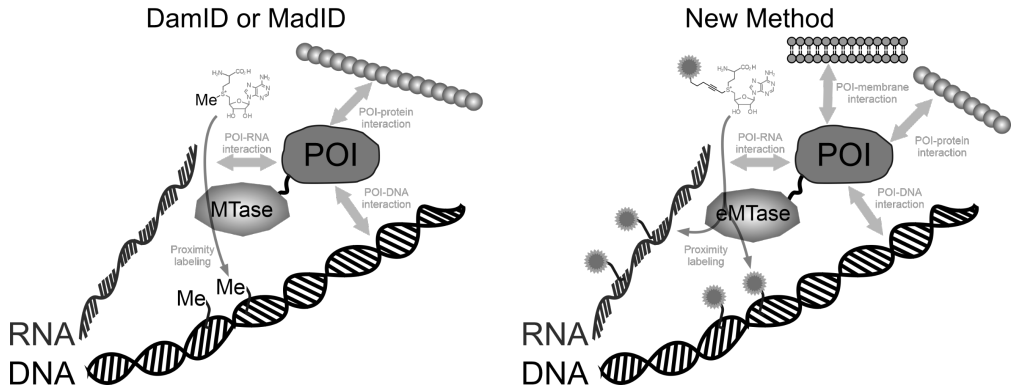

Figure 1: Comparison of traditional DamID/MadID approaches and the new engineered methyltransferase (eMTase) method for detecting protein–nucleic acid interactions. Unlike sequence-specific labeling with natural cofactors, the engineered enzyme uses SAM analogues to covalently tag both DNA and RNA in proximity to a protein-of-interest (POI), enabling stable, high-density labeling and detection via microscopy, sequencing, or optical mapping.

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The invention centers on an engineered, sequence-unspecific adenine-N6 methyltransferase (derived from M.EcoGII or homologues) modified by targeted amino acid substitutions (notably W46A, E44, C285, R336). These alterations enlarge or reshape the cofactor-binding pocket, enabling efficient use of SAM analogues bearing bulky functional groups in place of the native methyl group. Functional groups may include fluorophores, affinity handles, or chemically reactive tags.
When fused directly to a POI or indirectly targeted via antibody-protein A/G linkage, the engineered enzyme transfers its functional group to adenine bases in DNA and adenosine bases in RNA located near the POI. The covalent nature of the modification ensures signal stability during nucleic acid isolation, allowing subsequent visualization or mapping.
Detection can be achieved through:

- Fluorescence microscopy (wide-field, confocal, STED, MINFLUX) for in situ spatial resolution.
- Optical genome mapping to position labels within genomic coordinates.
- Direct modification-sensitive sequencing (Oxford Nanopore, PacBio) for precise identification of contact sites.

This approach uniquely enables simultaneous detection of protein-DNA and protein-RNA proximity, achieves high labeling density by discriminating against natural SAM, and adapts to multiple experimental contexts including fixed, permeabilized, or live-cell systems.

Advantages

- **Dual DNA and RNA compatibility** - enables simultaneous mapping of protein interactions with both nucleic acid types.
- **High labeling density** - engineered enzyme selectively uses SAM analogues, avoiding competition from natural cofactors.
- **Stable covalent tags** - labels withstand nucleic acid extraction, enabling robust downstream analysis.
- **Versatile detection options** - compatible with microscopy, optical mapping, and direct modification sequencing.
- **Sequence-unspecific activity** - not limited to recognition motifs, providing unbiased interaction profiling.

Potential applications

- **Gene regulation studies** - mapping transcription factors, chromatin remodelers, or RNA-binding proteins in situ.
- **Chromatin architecture analysis** - identifying nuclear compartment associations at high resolution.
- **RNA biology research** - visualizing protein-RNA complexes in the context of the transcriptome.
- **Epigenetics and modification profiling** - integrating protein proximity data with epigenomic maps.
- **Drug target validation** - assessing compound effects on protein-nucleic acid interactions in cells.

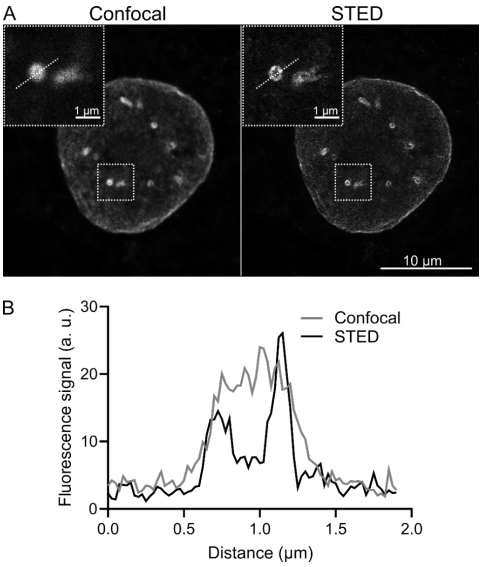

Figure 2: Super-resolution imaging of nucleic acids labeled by engineered EcoGII (W46A) methyltransferase targeted with antibody-protein A/G fusion. Confocal and STED microscopy show fluorescent labels deposited in proximity to lamin B1 at the nuclear periphery. The line intensity profile demonstrates improved spatial resolution and structural detail in STED compared to confocal imaging.

Patent Information

EP application (24.06.2025).

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