Not All Designer Nucleosomes are Created Equal: A Tale of Two Cysteines

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Designer nucleosomes are semi-synthetic nucleosomes incorporating specific histone post-translational modifications (PTMs). These reagents represent a powerful new technology - critical in understanding chromatin biology and for the development of novel drug targets and precision therapeutics. Of the two currently used synthetic methods, native chemical ligation (NCL) yields superior nucleosome preparations compared to methyllysine analog (MLA). Whereas MLA attempts to mimic the native methyllysine, NCL allows for incorporation of the actual PTM and is not limited to methyllysine.

CONCLUSION: Designer nucleosomes generated by NCL are far more reliable substrates for *in vitro* biochemical assays than those generated by MLA.

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Lysine methyltransferases and human disease

Methyltransferase enzymes are highly attractive therapeutic targets, as many are involved in the development of human diseases[1,2,3]. Nucleosomes are the fundamental repeating units of chromatin, consisting of approximately 147 base pairs of DNA wrapped around a histone octamer consisting of 2 copies each of the core histones H2A, H2B, H3 and H4[3,4]. Remarkably, this structure not only functions to efficiently package the genome but also regulates diverse cellular functions such as transcription, DNA repair, mRNA processing, and cellular differentiation[5,6,7]. These processes are controlled in part through reversible histone post-translational modifications (PTMs), which include methylation, acetylation, ubiquitination, and phosphorylation. PTM aberrations are associated with many human pathologies ranging from cancers to immunodeficiency disorders[8,9,10,11,12,13]. Therefore, the enzymes or effector-binding proteins that interact with chromatin to regulate the PTM landscape are compelling candidates for targeted drug development.

Lysine methylation of histones plays a critical role in regulating chromatin-mediated cellular processes and is associated with both transcriptional activation and repression[3]. These lysine residues can be either mono- (me1), di- (me2) or tri-methylated (me3) and importantly, these modifications regulate the affinity of effector proteins that impact chromatin structure and function. Analysis of the different histone methyllysine binding proteins as well as their functions in a chromatin context has historically been hampered by the lack of simple experimental tools.

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Current high throughput biochemical assays typically use modified synthetic peptides or recombinant histone octamers, however these substrates fail to accurately represent chromatin. Nucleosomes purified from biological sources provide a physiologically relevant substrate, but the PTM profiles of these products are highly heterogeneous, a feature that greatly complicates the use of these reagents in biochemical studies. By contrast, designer nucleosomes (dNUCs) provide the benefits of cell-derived nucleosomes, yet they also allow users to work with a homogenous PTM profile. This feature is essential for establishing reliable biochemical assays for drug discovery endeavors.

Feature of dNUC Method	NCL	MLA
Native histone sequence	Yes	No (Lys –> Cys mutation)
True methyllysine	Yes	No (Methylcysteine)
Multiple PTMs per protein	Yes	No
Multiple type of PTM per protein	Yes	No (lysine methylation only)

Table 1: Comparison of NCL and MLA methods

NCL is a superior method to generate designer modified nucleosomes

Competing chemical approaches have been developed to generate recombinant histones containing methylated residues for designer nucleosome assembly, including NCL and MLA[14,15,16]. Significantly, MLAs are structurally different from native methyllysine moieties. By contrast, NCL results in the scarless incorporation of native methyllysine. Comparison of NCL and MLA chemistries are shown in **Figure 1**.

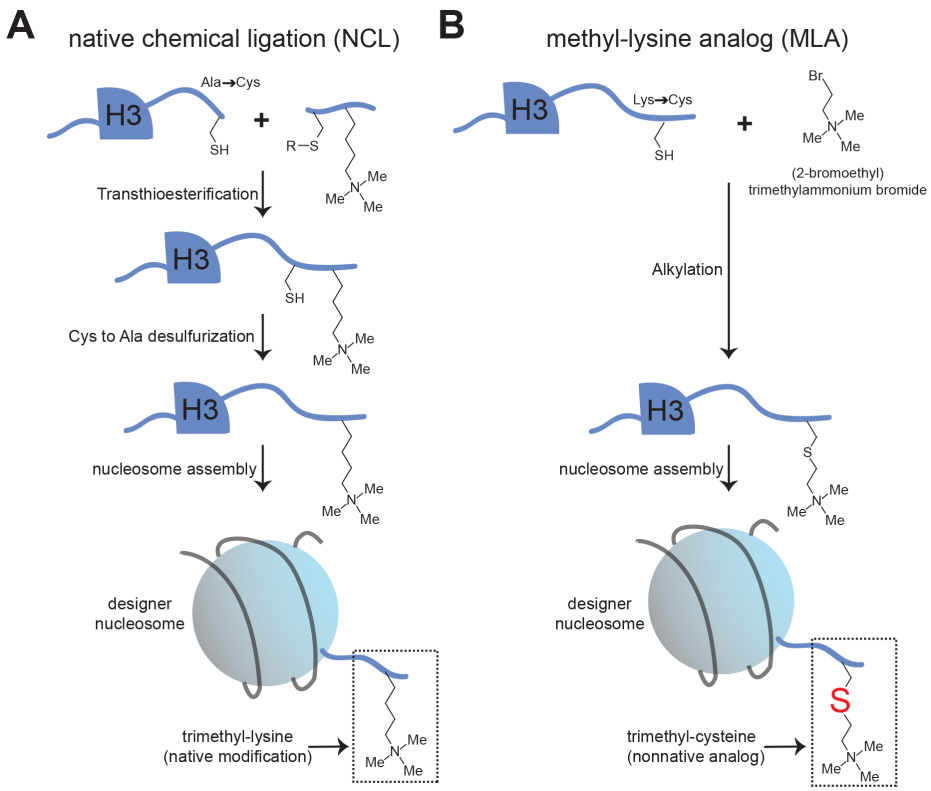

Figure 1: Comparison of NCL and MLA methods to generate methylated histones for designer nucleosomes assembly. (A) NCL uses a truncated histone protein to which a modified peptide is ligated to generate a modified full-length histone monomer (in this case trimethyllysine, but the peptide in question can contain any number or type of PTMs). This ligation requires an alanine to cysteine mutation at the ligation junction to facilitate linkage using a transthioesterication reaction. Following ligation, the cysteine is converted back to alanine via desulfurization to regenerate the native modified histone sequence, which can then be assembled into synthetic nucleosomes. (B) MLA generation requires a lysine to cysteine mutation at the desired methylation site. Using an alkylation reaction, a trimethyl moiety can be directed for covalent attachment to the cysteine residue. The resulting modified histone monomer can be assembled into designer nucleosomes. Significantly, MLA-modified histones carry trimethyl-cysteine. This resulting change in PTM identity dramatically alters the ability of proteins to interact with or catalyze this type of unnatural modification. Indeed, not all designer nucleosomes are created equal.

NCL and MLA nucleosomes are not biochemically equal

MLAs are distinct from their native counterparts in a number of chemical properties that can lead to altered specificity or affinity in effector binding assays[17,18,19]. Indeed, histone peptides bearing native methyllysines typically displayed 5-13 fold higher affinity than corresponding MLAs[20,21] when quantitatively assessed for binding affinity using cognate binding proteins. As well, the lens epithelium-derived growth factor (LEDGF) exhibits approximately 10,000-fold higher binding affin-

ity for H3K36me3 designer nucleosomes made by NCL over a H3K36me3 peptide. In contrast, H3K36me3 MLA nucleosomes only yielded a background level of binding[16]. Another disadvantage of employing the MLA method is that it is not compatible with the incorporation of different PTMs (e.g., H3S10p) or different degree of methylation states at multiple sites on the same histone (e.g., H3K9me2 and H3K27me3). Collectively, designer nucleosomes generated by NCL, and not those by MLA, are far more reliable and representative substrates for *in vitro* biochemical assays than their MLA counterparts.

Conclusion

Designer nucleosomes are semi-synthetic nucleosomes that incorporate specific histone post-translational modifications (PTMs). These reagents represent a powerful new technology that is poised to play a pivotal role in deciphering the fundamental principles of chromatin biology and the future development of novel drug targets and precision therapeutics[1,2]. However, methods employed to build designer nucleosomes dictate whether or not these reagents are reliable biochemical substrates. Native chemical ligation (NCL) and methyllysine analog (MLA) are two methods currently employed to generate core histone monomers containing specific PTMs, which can then be reconstituted into semi-synthetic nucleosomes (Figure 1). Whereas the MLA method represents a method that mimics the endogenous PTM, the NCL method allows for incorporation of native methyllysine (and many other PTMs) and provides far superior nucleosomes for PTM assay development and chromatin biology research.

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