Published in final edited form as:

J Am Chem Soc. 2010 March 3; 132(8): 2504–2505. doi:10.1021/ja909741q.

Approach to profile proteins that recognize post-translationally modified histone ‘tails’

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Abstract

Post-translational modifications (PTMs) of histones, proteins onto which DNA is packaged, are involved in many biological processes, including transcription, recombination and chromosome segregation. As these PTMs can be dynamic, combinatorial, and mediators of weak interactions, the comprehensive profiling all proteins that recognize histone PTMs is a daunting task. Here we describe an approach to design probes that can be used to identify proteins that directly interact with modified histones. Protein structure was used to guide the introduction of a photocrosslinker in the probe, so as to convert weak interactions into covalent linkages. The probe also included an alkyne group to facilitate click chemistry-mediated conjugation of reporter tags for the rapid and sensitive detection (via rhodamine) and affinity enrichment (via biotin) of labeled proteins. In particular, we developed and validated a probe that can selectively capture proteins that recognize trimethylated lysine-4 of histone H3 (H3K4me3) in whole proteomes. A complete profiling of H3K4me3 binding proteins should shed new light on cellular processes regulated by this PTM.

Protein post-translational modifications (PTMs) are involved in regulating many essential biological processes¹. There have been important advances in our ability to detect PTMs (e.g. phosphorylation or lipidation)¹. However, we currently lack reliable methods to identify, without bias, proteins that recognize PTMs, as PTMs can be dynamic, present on a small fraction of the cellular protein, or mediators of weak interactions. Recently, significant attention has focused on PTMs of histones, proteins on which DNA is packaged. Histone PTMs, such as acetylation, methylation, phosphorylation, ubiquitylation and sumoylation, are involved in regulation of basic cellular processes, including transcription, DNA repair, chromosome segregation and cell differentiation². It has also been proposed that histone PTMs may serve as a heritable epigenetic ‘code’³, which can transmit cellular information, not encoded at the level of DNA sequence, through cell division. The characterization of protein domains that recognize histones with different PTMs indicates that these non-covalent protein-protein interactions are relatively weak (μM). In addition to their chemical diversity, the PTMs are dynamic^{2,4} and combinatorial⁵, which can synergize or antagonize associations with different ‘readers’. To completely understand the roles of histone PTMs in different cellular processes, it is important to comprehensively profile all proteins that can recognize these PTMs.

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Supporting Information Available: Methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Here we report a robust strategy to identify proteins that directly interact with post-translationally modified histones.

Inspired by the successful applications of activity-based probes in protein profiling⁶, we used protein structure to guide the design of peptide probes that can convert weak non-covalent interactions into irreversible covalent linkages through photocrosslinking. The photocrosslinking groups were incorporated such that they are proximal to sites mediating the interaction, but are unlikely to perturb protein-protein association. The peptide probes also carry an alkyne group to facilitate 'click' chemistry-mediated conjugation of reporter tags for the rapid and sensitive detection (via rhodamine) or affinity enrichment (via biotin) of labeled proteins (Figure 1a). To develop and validate our approach, we first focused on trimethylation of lysine-4 of histone H3 (H3K4me3), an evolutionarily conserved and well-characterized PTM at the N-terminal region of the histone (so-called histone 'tail')⁷. H3K4me3 has been associated with regions of the genome that are transcriptionally active⁸. Known proteins that "read" the trimethylated H3K4 mark utilize different protein folds, including chromodomains, tudor domains or plant homeodomain (PHD) fingers⁹. Recent structures of these domains in complex with H3K4me3 peptides⁹ provided a basis for the design of our probes for capturing proteins that recognize this histone PTM.

As shown in Figure 1b, structural data reveals that PHD finger of ING2¹⁰, a known H3K4me3-binding protein, interacts with an H3K4me3 peptide mainly through residues between P-3 and P+2 (i.e. ARTK(me3)QT), relative to lysine-4 on histone-3. We therefore synthesized peptide probe **1** in which a benzophenone moiety replaced the side chain of Ala 7 (Figure 1c). We expected that the benzophenone radicals generated via UV-irradiation could be proximal to the binding protein but unlikely to interfere with the protein-protein interactions. The probe also included a spacer (H3 residues 8–15), and an alkyne for subsequent bio-orthogonal chemistry.

We examined the ability of probe **1** to covalently label the PHD finger of ING2 *in vitro*. The recombinant GST-fused PHD finger of ING2 (GST-ING2_{PHD}) was incubated with probe **1**, irradiated with UV light (1 h, optimizations shown in Figure S2), and coupled to rhodamine azide (Rh-N₃). Probe **1** successfully labeled the recombinant protein in a dose-dependent manner (Figure 2a). The labeling was saturated at ~1 μ M probe **1** (LC₅₀ = 0.3 μ M). Importantly, the labeling of the GST-ING2_{PHD} protein by probe **1** (1 μ M) can be competed by H3K4me3 peptide with an IC₅₀ = 2.8 μ M (Figure 2b), which is similar to the reported binding constant (K_d) of 1.5 μ M for the native peptide¹⁰. To study the ability of probe **1** to label other known H3K4me3-binding proteins, we tested the PHD finger of BPTF¹¹ and the double tudor domain of JMJD2A¹². The robust labeling of the both recombinant proteins was also achieved by probe **1** (Figure 3a). These data indicate that our probe is able to interact with distinct folds that recognize this PTM. As we showed for GST-ING2_{PHD}, the labeling of each of these two proteins can be competed by native H3K4me3 peptide. Importantly, this competition is specific, as the presence of either unmodified H3 peptide (H3K4me0) or H3 trimethylated at lysine-9 (H3K9me3) (Figure 3a) do not interfere with labeling.

To analyze whether probe **1** could be used to broadly profile H3K4-binding partners in complex proteomes, we first tested its ability to label GST-ING2_{PHD} that was added to HeLa cell lysates. We found that probe **1** (1 μ M) robustly labeled recombinant GST-ING2_{PHD} (10 μ g/mL, 280 nM) in cell lysates (2 mg/mL), and this labeling can also be competed by H3K4Me3 peptide (50 μ M) (Figure S3), consistent with our studies with recombinant protein alone. We next compared proteome labeling by **1** (1 μ M) in the absence or presence of H3K4Me3 peptide (50 μ M), without adding any recombinant protein. Using SDS-PAGE and in-gel fluorescence scanning, we were able to reproducibly detect differences in labeled proteins, as highlighted in Figure 3b (see Figure S4 for entire gel). As a final step in validating our method, we examined

if Probe **1** can specifically capture endogenous H3K4Me3-interacting partners. Probe **1** (2 μ M) was added either alone, or in the presence of H3K4me3 peptide (50 μ M), to HeLa cell lysates. These samples were then irradiated with UV light, treated with biotin azide (biotin- N_3) under click chemistry conditions, followed by streptavidin enrichment, SDS-PAGE and Western blot analysis. As shown in Figure 3c, endogenous ING2 was captured by the probe, and this association was competed by H3K4me3 peptide. Importantly, heterochromatin protein-1 (HP-1), a protein known to preferentially interact with H3K9me3 rather than H3K4Me3¹³, was not captured by the probe. Together these data indicate that our approach has the potential to identify H3K4Me3-binding proteins that have not been characterized thus far.

In conclusion, we have developed a peptide probe that is capable of capturing proteins that associate with histone H3K4 trimethylation in cellular contexts. By combining our approach with state-of-the-art mass spectroscopy, we should be able to profile proteins that recognize this, or other histone modifications. This approach should also be applicable to find ‘readers’ of combinatorial modifications linked to specific biological states (e.g. lysine-9 trimethyl and phosphorylated ser-10 of histone-3 during mitosis). In addition, this approach may be readily extended to profile proteins that recognize other PTMs, particularly when these modifications are dynamic or mediators of weak interactions. We are now working on comprehensively profiling proteins that recognize the H3K4 trimethyl mark. The findings from these studies will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Zafia Anklesaria for technical assistance and Lei Tan and Emily A. Foley for discussions. This work was supported by the NIH (GM65933). X. Li was supported by C. H. Li Memorial Scholar Fund Postdoctoral Fellowship.

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Figure 1.

(a) Strategy to capture proteins that recognize histone PTMs. (b) Structure of PHD finger of ING2 binding to a H3K4me3 peptide. (c) Chemical structure of probe 1.

Figure 2.

(a) The concentration-dependent labeling of purified PHD finger of ING2 by probe **1**. (b) H3K4me3 peptide inhibited the labeling of PHD finger by probe **1** (1 μ M). The probe-labeled GST-tagged PHD finger of ING2 (100 ng, 280 nM) was detected via conjugation to a rhodamine-azide tag, followed by SDS-PAGE analysis, and in-gel fluorescence scanning. Each data point corresponds to the average of two independent trials.

Figure 3.

(a) The labeling of recombinant H3K4Me3-binding proteins (100 ng) by **1** (1 μ M) was selectively inhibited by H3K4me3 peptide (30 μ M) but unaffected by unmodified H3 (H3K4me0) peptide (30 μ M) and H3K9me3 peptide (30 μ M). (b) Probe **1** (1 μ M) labeled proteins in cell lysates (2 mg/mL). Differences in proteins labeled by probe in the absence and presence of H3K4Me3 peptide (50 μ M) are highlighted by arrowheads (for entire gel image, see Figure S4). (c) Probe **1** selectively captured endogenous ING2 from cell lysates (for loading control, see the Supporting Information Figure S5).