

Molecular Modeling of Differentially Phosphorylated Serine 10 and Acetylated lysine 9/14 of Histone H3 Regulates their Interactions with 14-3-3 ζ , MSK1, and MKP1

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Abstract: Histone modifications occur in precise patterns, with several modifications known to affect the binding of proteins. These interactions affect the chromatin structure, gene regulation, and cell cycle events. The dual modifications on the H3 tail, serine10 phosphorylation, and lysine14 acetylation (H3Ser10PLys14Ac) are reported to be crucial for interaction with 14-3-3 ζ . However, the mechanism by which H3Ser10P along with neighboring site-specific acetylation(s) is targeted by its regulatory proteins, including kinase and phosphatase, is not fully understood. We carried out molecular modeling studies to understand the interaction of 14-3-3 ζ , and its regulatory proteins, mitogen-activated protein kinase phosphatase-1 (MKP1), and mitogen- and stress-activated protein kinase-1 (MSK1) with phosphorylated H3Ser10 alone or in combination with acetylated H3Lys9 and Lys14. In silico molecular association studies suggested that acetylated Lys14 and phosphorylated Ser10 of H3 shows the highest binding affinity towards 14-3-3 ζ . In addition, acetylation of H3Lys9 along with Ser10PLys14Ac favors the interaction of the phosphatase, MKP1, for dephosphorylation of H3Ser10P. Further, MAP kinase, MSK1 phosphorylates the unmodified H3Ser10 containing N-terminal tail with maximum affinity compared to the N-terminal tail with H3Lys9AcLys14Ac. The data clearly suggest that opposing enzymatic activity of MSK1 and MKP1 corroborates with non-acetylated and acetylated, H3Lys9Lys14, respectively. Our in silico data highlights that site-specific phosphorylation (H3Ser10P) and acetylation (H3Lys9 and H3Lys14) of H3 are essential for the interaction with their regulatory proteins (MKP1, MSK1, and 14-3-3 ζ) and plays a major role in the regulation of chromatin structure.

Keywords: modeling, histone H3 modifications, 14-3-3 ζ , MSK1, MKP1

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Introduction

Histones undergo different posttranslational modifications such as lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, sumoylation, ADP-ribosylation, and ubiquitination.¹ These modifications are read by the binding partner 'readers' at the level of a distinct or multiple modifications. The binding of partners to histones facilitate chromatin organization to control gene expression and perform particular biological functions in specific phases of the cell cycle.²⁻⁴

Phosphorylation at serine10 on histone H3 (H3Ser10P) is linked with two different events, including transcriptional activation in G1-phase and chromatin condensation in G2/M-phase of the cell cycle.⁵ Literature suggests that the phospho-specific domain containing protein, 14-3-3 ζ , an isoform of 14-3-3 family interacts, with H3Ser10P.⁶ The crystal structure of 14-3-3 ζ with H3Ser10P peptide (PDB: 2C1N) indicates that it is stabilized by intermolecular hydrogen bonding interactions.⁶ The side chains of the 14-3-3 ζ residues Lys120, Asn224, and Asn173 interact with the backbone carbonyl and amide groups of the H3 peptide. Phosphorylated H3Ser10 is neutralized by a basic pocket formed by 14-3-3 ζ with the highly basic amino acids Lys49, Arg127, Arg56, and the side chain amide nitrogen of Asn173. It has also been shown that 14-3-3 ζ proteins bind to 'writers' and 'erasers' of histone modification, such as histone acetyltransferase (HAT) and histone deacetylases (HDAC) and help in mediating transcriptional activation of specific subset of mammalian genes and DNA replication mediated through acetylation on Lys9 and Lys14.⁷⁻⁹ Further, the crystal structure of 14-3-3 ζ interaction with H3Lys9AcSer10P peptide (PDB: 2C1J) showed hydrogen bonding interactions of Ser10P with triad Arg56, Arg127 and Tyr128 and Lys9Ac with Asn224.⁶ Earlier studies have also linked H3Ser10P to Lys14Ac on the same histone tail, and it has been shown that H3Ser10PLys14Ac is important for binding of 14-3-3 ζ with high affinity compared to H3Ser10P alone.^{9,10} The bromodomain-containing proteins are known to interact with acetylated histones;¹¹ 14-3-3 ζ does not contain a bromodomain, suggesting that its molecular interaction is specific to the phosphoserine residue. Therefore, whether dual acetylation marks at the chromatin, H3Lys9, or H3Lys14 is important for stabilizations

and protection of the phosphorylated H3Ser10 during transcriptional activation of immediate early genes in interphase of the cell cycle remains an enigma.

The phosphorylation of H3Ser10 on the promoter of immediate early genes in response to epidermal growth factor (EGF) is mediated through mitogen- and stress-activated kinase1 (MSK1), whereas dephosphorylation of H3Ser10P occurs by mitogen-activated protein kinase phosphatase 1 (MKP1) in response to vascular endothelial growth factor (VEGF).^{12,13} MKP1 is also known to be overexpressed in many human tumors.^{14,15} Thus, MKP1 is a reasonable target for the treatment of cancer, but the search for inhibitors has been difficult because of the non-availability of structural information for MKP1. MKP1 is a 367-amino acid protein consisting of two domains, inactive N-terminal rhodanase domain and active C-terminal phosphatase domain, separated by a flexible loop region containing six proline residues.¹⁶ MSK1, a kinase that phosphorylates H3Ser10, is an 802-amino acid protein with a serine/threonine nuclear kinase domain and acts downstream of both ERK and p38 MAPKs.^{17,18} MSK1 contains two kinase domains separated by a flexible loop segment. The crystal structure of the MSK1 N-terminal domain is available (PDB ID: 1VZO 24-345); however, inactive MSK1 requires the association of an activation loop residue (Ser376) phosphorylation for conversion into the active form.¹⁹

Inducible site-specific H3 phosphoacetylation (H3Ser10PLys9AcLys14Ac) is a tightly regulated process that occurs on the nucleosomes, which is well-established in the literature.¹² It is not clear whether such dynamic chromatin markers, phosphorylation, and acetylation on H3 tails, are required independently or in concert with one another for their interaction with 14-3-3 ζ and MKP1. Additionally, whether acetylation is a pre-requisite for MSK1-mediated phosphorylation of H3Ser10 is not known. The investigation of interactions between H3Ser10P along with Lys9 and Lys14 modification(s) and their binding partner, 14-3-3 ζ , has been carried out using biochemical methods. The existing biochemical experiments from different labs on different cell lines in response to different inducible agents support and contradict both hypotheses.^{6,9} The theoretical efforts to delineate the cross-talk and understand the molecular association of phosphorylated H3 or acetylated H3 singly or in different combinations (H3Ser10PLys9AcLys14Ac)

with ‘writers’ and ‘erasers’ have been limited. The three-dimensional structures of MKP1 and MSK1 also remains unknown, which are essential for understanding their conformation, active regions, interaction sites, and binding domains with other proteins. To study the molecular interactions of histone H3 with its binding partner, the expert interface of Haddock docking server was used. Haddock is an information-driven flexible docking approach, where active sites residues are provided for docking.²⁰ To ascertain the role of each modified residues of histone H3, different combination of modified residue complexes were built and docked. The Haddock score of docked protein complex is a weighted sum of intermolecular electrostatic, van der Waals, desolvation, and buried surface area terms.

Molecular modeling studies suggest that the differential modification(s), Lys9Ac, Ser10P, and Lys14Ac at the N-terminal tail of histone H3 regulates its interaction with 14-3-3 ζ , MSK1, and MKP1. In agreement with an earlier study, our *in silico* results also suggest that dual phospho-acetylation of H3Ser10-PLys14Ac favors the interaction with 14-3-3 ζ . The MKP1 interaction with N-terminal tail of H3 showed a strong preference for Ser10PLys14AcLys9Ac modifications over Ser10P alone. The interphase-specific H3 kinase, MSK1 phosphorylates H3Ser10 with the highest binding affinity when Lys9Lys14 are non-acetylated. Our *in silico* studies demonstrate that differential phospho-acetylation on the N-terminal tail of H3 is highly dynamic and regulates its reversible interaction(s) with binding partners, MSK1 and MKP1.

Methods

Homology modeling of MKP1 and MSK1 structures

We conducted homology modeling due to the unavailability of crystallographic coordinates of MKP1. The model of active the C-terminus phosphatase domain of MKP1 (172–314 aa) was constructed using PDB ID: 3EZZ as a template. The N-terminal crystal structure of MSK1 is available, but to model the phosphorylated Ser376 residue of the flexible loop near the active site, PDB ID: 3A8X was used as a template for homology modeling of MSK1 with amino acid residues from 42–380. Modeling studies were performed using Swiss Model.²¹ The modeled structure of

MKP1 and MSK1 were cross-validated using multiple tools such as Procheck,²² Verify_3D,²³ and Errat²⁴ using SAVES server (<http://nihserver.mbi.ucla.edu/SAVES/>). The cross-validated modeled structures of MKP1 and MSK1 are shown as Figure 1 and secondary structures for both are depicted in Supplementary Figure S1.

Refinement of crystal structure of 14-3-3 ζ with native H3 peptide and its posttranslational modifications (PTM)

In the crystal structure of histone H3 (PDB ID: 2C1J),⁶ acetylation at Lys9 and Lys14 and phosphorylation at Ser10 was generated using Discovery Studio Visualizer version 3.5. A total of seven structures of histone H3 were modeled which encompassed combinations of modifications [Lys9, Ser10, Lys14, Lys9-Ser10, Lys9-Lys14, Ser10-Lys14, and Lys9-Ser10-Lys14] (Supplementary Fig. S2). The Haddock server²⁰ was used to score the refinement of PDB ID: 2C1J as native and the modified 14-3-3 ζ and histone H3 complex. The Haddock scores of the complexes are tabulated in Table 1 and Figure 2.

Molecular association of MSK1 with histone H3 and its PTM modified structure

The full-length (1–21 aa) loop structure of histone H3 was used to build the three PTM-modified structure [Lys9, Lys14, Lys9-Lys14]. The modeled structure of MSK1 was docked with native and the three PTM-modified structure of histone H3 using the Haddock

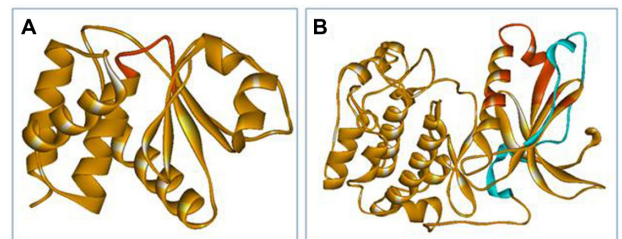


Figure 1. Homology modeling of MKP1 and MSK1 structures (A) Homology modeled structure of C-terminal phosphatase domain of MKP1 (172–314 aa) is shown in yellow color, while the active site includes His257, Cys258, Gln259, Ala260, Gly261, Ile262, Ser263, and Arg264 residues in orange color. (B) Homology modeled structure of N-terminal kinase domain of MSK1 (42–380 aa) is shown in yellow color. The blue colored region is the flexible loop near the active site. The orange color indicates the active site residues Lys85, Ile88, Val89, Thr95, Arg102, Gln122, and Leu127.

**Table 1.** 14-3-3 ζ with histone H3 PDB complex and its PTM structure.

PDB complexes	HADDOCK score	Interacting residues	
		H-bonds	Hydrophobic
14-3-3 ζ H3	-40.3 \pm 2.1	Arg8: <i>Glu180</i> Lys9: <i>Asp223, Asn224</i> Thr11: <i>Lys120, Asn173</i> Lys14: <i>Lys49</i>	Ala7: <i>Leu227</i> Ser10: <i>Leu172</i> Gly12: <i>Lys49</i>
14-3-3 ζ H3Ser10P	-62.4 \pm 5.3	Arg8: <i>Glu131, Glu180</i> Lys9: <i>Asp223, Asn224</i> Ser10: <i>Arg56, Arg127, Tyr128</i> Thr11: <i>Lys120, Asn173</i> Gly12: <i>Arg56</i> Lys14: <i>Lys49</i>	Arg8: <i>Leu227</i> Lys9: <i>Leu220</i> Ser10: <i>Leu172, Val176</i>
14-3-3 ζ H3Ser10PLys9Ac	-46.5 \pm 2.6	Arg8: <i>Glu180</i> Lys9: <i>Asn224</i> Ser10: <i>Arg56, Arg127, Tyr128</i> Thr11: <i>Lys120, Asn173</i> Gly13: <i>Lys49</i>	Ala7: <i>Leu227</i> Lys9: <i>Asp223</i> Ser10: <i>Leu172</i>
14-3-3 ζ H3Ser10PLys14Ac	-119.7 \pm 2.5	Ala7: <i>Asp223</i> Arg8: <i>Glu131, Glu180</i> Lys9: <i>Asp223, Asn224</i> Ser10: <i>Arg56, Arg60, Arg127</i> Thr11: <i>Lys120</i> Gly12: <i>Lys49</i>	Ala7: <i>Leu227, Leu220</i> Lys9: <i>Leu220</i> Thr11: <i>Lys120</i>
14-3-3 ζ H3Lys9AcSer10PLys14Ac	-54.0 \pm 6.8	Arg8: <i>Glu180</i> Lys9: <i>Asn224</i> Ser10: <i>Arg56, Arg127, Tyr128</i> Thr11: <i>Asn173, Lys120</i> Gly13: <i>Lys49</i>	Ala7: <i>Leu227</i> Arg8: <i>Val176</i> Ser10: <i>Leu172</i>
14-3-3 ζ H3Lys9Ac	-29.0 \pm 4.5	Arg8: <i>Glu180</i> Lys9: <i>Asn224</i> Thr11: <i>Lys120, Asn173</i> Lys14: <i>Lys49</i>	Ala7: <i>Leu227, Val176</i> Lys9: <i>Asp223</i> Ser10: <i>Asn173</i>
14-3-3 ζ H3Lys14Ac	-98.9 \pm 2.4	Ala7: <i>Asp223</i> Arg8: <i>Glu180</i> Lys9: <i>Asp223, Asn224</i> Thr11: <i>Asn173, Lys120</i> Gly12: <i>Lys49</i> Lys14: <i>Arg60</i>	Ala7: <i>Leu227</i> Lys9: <i>Leu220</i> Arg8: <i>Glu180</i> Gly12: <i>Lys49</i>
14-3-3 ζ H3Lys9AcLys14Ac	-43.0 \pm 1.8	Arg8: <i>Glu180</i> Lys9: <i>Asn224</i> Ser10: <i>Arg127</i> Thr11: <i>Lys120, Asn173</i> Gly13: <i>Lys49</i>	Lys9: <i>Leu220, Leu227</i> Arg8: <i>Asp223</i> Gly12: <i>Lys49</i>

Note: Bold letters indicate histone H3 and italics indicate binding partners.

server (Fig. 1B and Supplementary Fig. S3).²⁰ The active site residues (Lys85, Ile88, Val89, Thr95, Arg102, Gln122, and Leu127) of MSK1 and (Lys9, Ser10, and Lys14) of histone H3 were provided as input parameters for targeted docking. The protein-protein interactions, Haddock score, hydrogen bonds, and hydrophobic interactions are tabulated in Table 2 and Figure 3.

The molecular interactions of the docked complexes were analyzed using Ligplot²⁵ for hydrophobic interactions (Supplementary Fig. S4). Discovery studio visualizer 3.5 was used to identify residues involved in hydrogen bonding between the two proteins and for the diagrammatic illustration of the residues involved in the hydrogen bond formation in the docked complex.

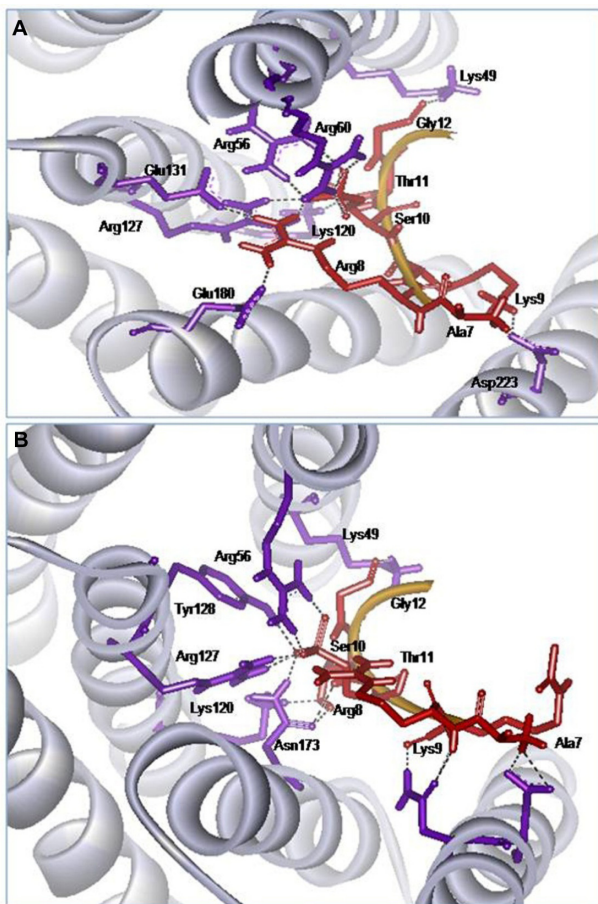


Figure 2. Docking of 14-3-3- ζ with histone H3 and its PTM structure (A) 14-3-3- ζ and histone H3Ser10Lys14Ac (B) 14-3-3- ζ and histone H3Lys9AcSer10Lys14Ac. The ribbon diagram of 14-3-3- ζ (grey) with the residues (violet) and histone H3 peptide (orange) with the residues (red) are involved in H-bonding interaction and are shown as a dotted black line.

Molecular association of native MKP1 with histone H3 and its PTM modified structure

The full length (1–21 aa) loop structure of histone H3 from the crystal structure of the *Xenopus* nucleosome (PDB ID: 1KX5) was used.²⁶ Histone H3 is 100% conserved between *Xenopus* and humans. The combination of Lys9, Ser10, and Lys14 modifications was generated by site-specific acetylation and phosphorylation resulting in seven PTM structures of histone H3 [Lys9, Ser10, Lys14, Lys9-Ser10, Lys9-Lys14, Ser10-Lys14, and Lys9-Ser10-Lys14]. The modeled structure of MKP1 was docked with native and seven PTM structures of histone H3 using the Haddock server (Fig. 1A and Supplementary Fig. S3).²⁰ The active site residues (His257, Cys258, Gln259,

Ala260, Gly261, Ile262, Ser263, and Arg264) of MKP1 and (Lys9, Ser10, and Lys14) of histone H3 were used as input parameters for docking. The Haddock scores of the complexes are tabulated in Table 3 and Figure 4. The results were analyzed for protein-protein interactions and emphasis was given for weak intermolecular interactions such as hydrogen bonding and hydrophobic interactions.

Results and Discussion

Histone H3 phosphorylation is involved in the transcriptional activation of genes in interphase cells. The phosphorylation of histone H3Ser10 is known to facilitate acetylation of Lys9 and Lys14. Cross-talk between site-specific phosphorylation and acetylation of the H3 tail regulates this process, but whether this occurs singly or in combination is not clearly understood. We have investigated *in silico* interactions between the regulatory proteins MKP1, MSK1, and 14-3-3 ζ and different combination of site-specific histone H3 modifications on Lys9, Ser10, and Lys14.

Homology modeling of MKP1 and MSK1 structures

Homology modeling was carried out for MKP1 and MSK1 by studying their molecular interactions with modified and unmodified H3.

Modeling of MKP1 structure

MKP1 consists of 367 amino acids with two domains that are separated by a flexible loop region. Truncated MKP1 with a deleted N-terminal rhodanase domain showed evidence of better phosphatase activity than its full-length counterparts, both *in vivo* and *in vitro*.²⁷ However, C-terminal truncations could neither change substrate affinity nor substrate-dependent catalytic activation. These studies clearly suggest that the C-terminal domain regulates the phosphatase activity of MKP1.²⁸ To examine the importance of MKP1 protein and its molecular interactions with other proteins, we built conducted modeling studies as the crystal or solution structure of MKP1 was not available. Data from the Protein Data Bank revealed that the C-terminal crystal structure of human MKP2 (PDB ID: 3EZZ) has sequence identity of 85.31% with human MKP1. Therefore, this structure was used

**Table 2.** Docking of native MSK1 with histone H3 and its PTM structure.

Docked complexes	HADDOCK score	Interacting residues	
		H-bonds	Hydrophobic
MSK1 H3	-92.1 ± 7.8	Ser10: <i>Lys43, Val44</i> Gly12: <i>Lys85</i> Lys14: <i>Glu124, Leu364, Glu369</i> Arg17: <i>Lys370</i> Leu20: <i>Lys370</i> Ala21: <i>Lys370</i>	Gly12: <i>Thr123</i> Gly13: <i>Glu369</i> Lys14: <i>Glu369</i>
MSK1 H3Lys9Ac	-47.4 ± 9.0	Ala1: <i>Glu369</i> Arg8: <i>Glu47</i> Lys9: <i>Glu124</i> Ser10: <i>Thr125, Lys126</i> Gly12: <i>Glu47</i> Arg17: <i>Tyr359</i>	Lys9: <i>Glu124</i> Ser10: <i>Lys126</i> Thr11: <i>Glu47, Tyr359</i>
MSK1 H3Lys14Ac	-64.0 ± 6.5	Ala1: <i>Ser367, Glu369</i> Thr3: <i>Glu124</i> Lys9: <i>Glu124, Glu369</i> Ser10: <i>Lys85, Glu369</i> Arg17: <i>Lys43, Glu47</i> Lys18: <i>Glu47, Ile69</i>	Arg2: <i>Pro365</i> Gly13: <i>Glu42</i>
MSK1 H3Lys9AcLys14Ac	-50.2 ± 10.2	Ala1: <i>Glu369</i> Arg8: <i>Glu47</i> Lys9: <i>Glu124</i> Ser10: <i>Thr125, Lys126</i> Gly12: <i>Glu47</i> Arg17: <i>Tyr359</i>	Arg2: <i>Pro365</i> Ser10: <i>Thr123</i> Thr11: <i>Tyr359</i>

Note: *Bold letters indicate histone H3 and italics indicate binding partners.

as a template for homology modeling of C-terminal of MKP1 from 172–314 amino acids. The dual specificity protein phosphatase (DUSP) domain is also reported to be located between 180–312 amino acids. The modeled structure of MKP1 was found to have a QMEAN Z-score of -0.693.²¹ The energy of the MKP1 structure was minimized using Discovery studio 2.5, from an initial potential energy of -5246.07 to -9131.51 kcal/mol by conjugate gradient converging at 1397 steps. The MKP1 structure was later validated using the SAVES server and showed that 0.8% residues were located in the disallowed region of the Ramachandran plot according to Procheck,²² confirming the correct overall geometry of the MKP1 model. Verfy_3D²³ showed 89.19% residues with average 3D to 1D scores >0.2 by assigning a structural class. The modeled structure of C-terminal of MKP1 (172–314 aa) and the active site residues (257–264 aa) are shown in red color in Figure 1A and Supplementary Figure S1A. The modeled structure has four beta-sheets, five alpha-helices, and seven loops. The overall quality of the model is given by

ERRAT score²⁴ of 98.519 by analyzing the non-binding interaction between different atom types.

Modeling of MSK1 structure

Earlier studies showed that the C-terminal (CTD) and N-terminal domains (NTD) of MSK1 are structurally distinct kinases. NTD MSK1 belongs to the AGC kinase family and is characterized by a kinase domain followed by a hydrophobic motif region with phosphorylation sites.¹⁷ The crystal structure of the N-terminal kinase domain of MSK1 is available (PDB ID: 1VZO 24–345); however, inactive MSK1 requires an association with the phosphorylated loop residue (Ser376) to convert into the active form. Therefore, to model the loop with the N-terminal domain, PDB ID: 3A8X was used as template for homology modeling. 3A8X is the crystal structure of protein kinase C (PKC- ι), which has sequence identity of 62% with the N-terminus of MSK1. The modeled structure of MSK1 has a QMEAN Z-score -1.216.²⁰ The modeled structure of N-terminal of MSK1 (42–380 aa) with the active site residues (Lys85, Ile88, Val89, Thr95, Arg102,

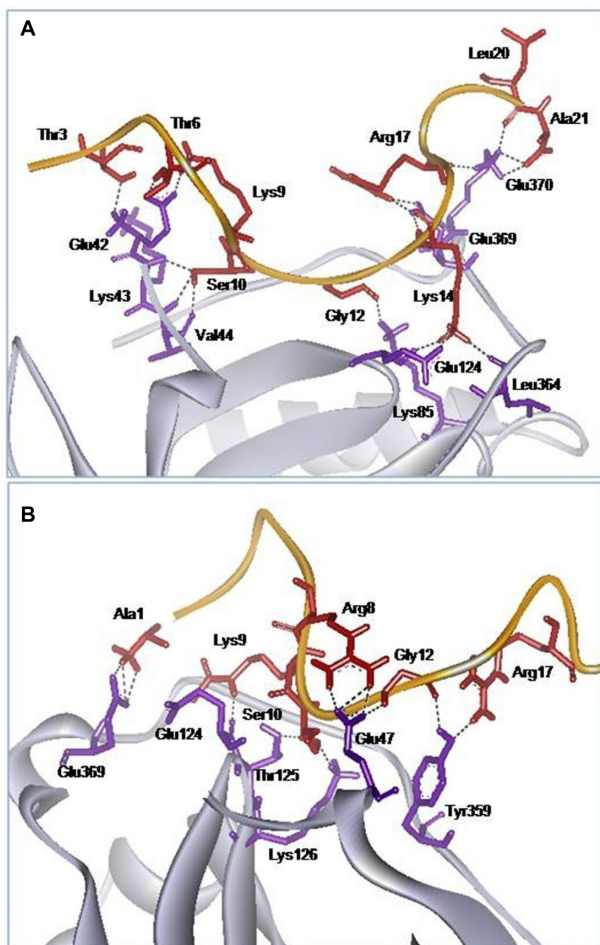


Figure 3. Docking of MSK1 with histone H3 and its PTM structure (A) MSK1 and histone H3 and (B) MSK1 and histone H3Lys9AcLys14Ac. The ribbon diagram of MSK1 (grey) with the residues (violet) and histone H3 peptide (orange) with the residues (red) are involved in the H-bonding interaction and is shown as a dotted black line.

Gln122, and Leu127) are highlighted in red color, while the cyan color indicates the loop (345–380 aa) in Figure 1B. The modeled structure was energy minimized by Discovery studio 2.5 from an initial potential energy of -11978.41 to -22806.55 kcal/mol by conjugate gradient, which converged at 1348 steps. The modeled structure has four beta-sheets, fifteen alpha-helices, and fifteen loops (Supplementary Fig. S1B). The MSK1 model validated through the SAVES server showed 0.7% residues are in the disallowed region of the Ramachandran plot according to Procheck,²² which confirmed the correct overall geometry. Verfy_3D²³ showed that 88.82% residues have average 3D to 1D scores of >0.2 by assigning a structural class. The overall quality of the model was given by the ERRAT score²⁴ of 95.886 by analyzing the non-bonding interaction between different atom types.

The modeled structures of MSK1 and MKP1 with active domains will be used for molecular interaction studies with H3 peptide with either phosphorylation or acetylation at specific residues, Lys9, Ser10, or Lys14.

Interaction of crystal structure of 14-3-3 ζ with native H3 peptide and its PTM modified structures

Phosphorylation of histone H3 at Ser10 has been implicated in transcriptional activation of immediate early genes in organisms ranging from yeast to humans in interphase.²⁹ Mahadevan et al demonstrated that 14-3-3 ζ interacts with H3 only when phosphorylated at Ser10.⁶ Additionally, recruitment of 14-3-3 ζ has been observed at the promoters of transcriptionally active genes, *c-fos* and *c-jun*.⁶ The activation of HDAC1 gene transcription and binding of 14-3-3 ζ at its promoter have been shown to be directly correlated with phosphorylation of H3Ser10.⁹ 14-3-3 ζ has also been shown to play a crucial role in the transcription of the mammalian FOSL1 gene by binding of the histone acetyltransferase, MOF.³⁰ The study also suggested that H3Lys9Ac is involved in recruitment of MOF, but supportive evidence and how 14-3-3 ζ mediates the crosstalk between H3Ser10 phosphorylation and Lys9 acetylation during transcription are not available.

The interaction of 14-3-3 ζ with phosphorylated proteins occurs through the two most favorable binding motifs.³¹ The phosphorylated peptide of H3 forms a conserved primary interaction with Arg56, Arg127, and Tyr128 residues of 14-3-3 ζ . Since the crystal structure of 14-3-3 ζ bound to an H3 peptide was available (PDB ID: 2C1J), the complex was subjected to the refinement mode of the Haddock server to score the interactions (Table 1 and Supplementary Fig. S4.1). The complex in which H3 is modified at Ser10 and Lys14 showed a high Haddock score, while the complex with acetylation at Lys9 showed the lowest Haddock score. In all the complexes with a phosphorylated Ser10, a conserved interaction with triad Arg56, Arg127, and Tyr128 was observed (Fig. 2). Our docking studies also support earlier studies of the molecular interaction between the phosphopeptide-interacting motif and the Arg–Arg–Tyr triad of 14-3-3 ζ .³²

Our in silico data suggest that 14-3-3 ζ interacts more strongly with H3Ser10P than with H3Lys9AcSer10P,

**Table 3.** Docking of native MKP1 with histone H3 and its PTM structure.

Docked complexes	HADDOCK score	Interacting residues	
		H-bonds	Hydrophobic
MKP1 H3	-61.8 ± 6.8	Arg8: <i>Gln259</i> Thr11: <i>Asp227</i> Lys14: <i>Asn228</i> Arg17: <i>Asp227, Asn228</i> Ala21: <i>His229</i>	Ser10: <i>Ala260</i> Lys14: <i>Asn228</i>
MKP1 H3Ser10P	-90.3 ± 3.8	Arg8: <i>Gln259</i> Ser10: <i>Cys258, Gln259, Ala260, Gly261, Ile262, Ser263, Arg264</i> Arg17: <i>Asn298</i>	Thr3: <i>Ile294</i> Lys9: <i>Ser296</i>
MKP1 H3Lys9AcSer10P	-121.6 ± 16.4	Lys9: <i>Gln259</i> Thr11: <i>Asp227</i> Ser10: <i>Cys258, Gln259, Ala260, Gly261, Ile262, Ser263, Arg264</i>	Gly13: <i>Phe299</i> Lys14: <i>Phe299</i> Lys18: <i>Phe299</i>
MKP1 H3Ser10PLys14Ac	-122.6 ± 17.7	Thr3: <i>Arg292</i> Thr11: <i>Asp227, Asn298</i> Arg17: <i>Ser296, Pro297</i> Lys18: <i>Asp282, Glu286</i> Ser10: <i>Cys258, Gln259, Ala260, Gly261, Ile262, Ser263, Arg264</i>	Lys9: <i>Ser185</i> Lys14: <i>Phe299</i>
MKP1 H3Lys9AcSer10PLys14Ac	-138.6 ± 12.1	Thr11: <i>Asp227</i> Ser10: <i>Gln259, Ala260, Gly261, Ile262, Ser263, Arg264</i> Arg17: <i>Ser296, Pro297</i> Lys18: <i>Asp282, Glu286</i>	Lys9: <i>Ile262, Ala260</i> Gly13: <i>Phe299</i> Lys14: <i>Phe299, Asn298</i> Lys18: <i>Phe285</i>
MKP1 H3Lys9Ac	-56.8 ± 6.9	Ala1: <i>Ser296</i> Thr3: <i>Asp227</i> Lys9: <i>Gly261</i> Ser10: <i>Arg292</i> Lys14: <i>Tyr187</i> Lys18: <i>His213</i>	Lys4: <i>Asp227</i> Lys9: <i>Ala260, Ile262</i> Gly13: <i>Tyr187</i>
MKP1 H3Lys14Ac	-67.8 ± 6.3	Ala1: <i>Asn209, Glu226</i> Thr3: <i>Ser185, Tyr187</i> Arg8: <i>Asp227, Gln259</i> Lys9: <i>Gln259</i> Ser10: <i>Asp227</i>	Lys4: <i>Tyr187</i> Lys9: <i>Ala260</i> Thr11: <i>Asp227</i>
MKP1 H3Lys9AcLys14Ac	-61.9 ± 4.4	Ala1: <i>Ser296</i> Thr3: <i>Arg264</i> Lys9: <i>Gly261, Ile262</i> Lys14: <i>Tyr187</i> Lys18: <i>Ser190, His213</i>	Lys9: <i>Gln259, Ile262, Ser263</i> Arg17: <i>Tyr187</i>

Note: *Bold letters indicate histone H3 and italics indicate binding partners.

which contradicts previous experimental results.^{9,33} A possible reason for the lower binding affinity of H3Lys9AcSer10P with 14-3-3ζ is the change in the salt bridge and H-bonding between the two proteins (Supplementary Fig. S4.1 and S4.1e). The acetylation at Lys9 and Lys14 alters the specificity of interaction of the phosphorylated peptide. Specifically, acetylation of Lys9 confers a negative charge, which prevents salt-bridge formation with Asp223 observed in all the complexes. Acetylated Lys9 prefers hydrogen

bonding with Asn224 rather than Asp223. However, non-acetylated Lys9 forms salt-bridge interaction with Asp223 and the hydrogen bond with Asn224. This suggests that the non-acetylated peptide at Lys9 may bind with higher affinity to 14-3-3ζ compared to the peptide with an acetylated Lys9. In the crystal structure, the side-chain of Lys14 pointing away from 14-3-3ζ thus limits a direct interaction. To compare the individual role of acetylation at Lys9 and Lys14, the complex with only Lys14 acetylated yielded -98.9

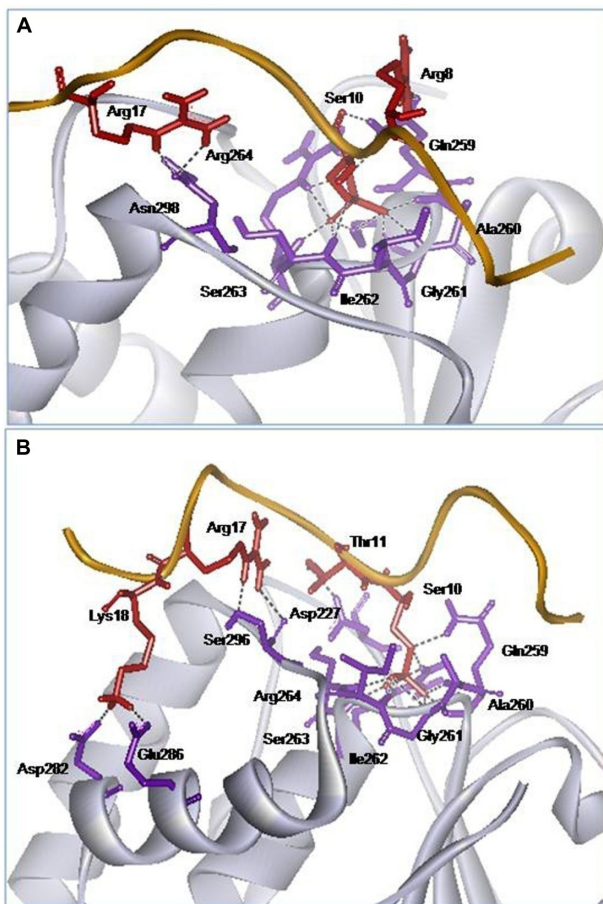


Figure 4. Docking of MKP1 with histone H3 and its PTM structure (A) MKP1 and histone H3Ser10 and (B) MKP1 and histone H3Lys9Ac-Ser10Plys14Ac. The ribbon diagram of MKP1 (grey) with the residues (violet) and histone H3 peptide (orange) with the residues (red) are involved in the H-bonding interaction and is shown as a dotted black line.

Haddock score as compared to -29.0 score from only Lys9 acetylated complex. To study the effect of Lys9 and Lys14 acetylation in combination with Ser10 phosphorylation, the complex with Ser10 and Lys14 modification yielded highest score -119.7 as compared to -46.5 for the complex with Ser10 and Lys9 modification (Table 1).

The complex with all modifications yielded a lower score compared to the 14-3-3 ζ H3Ser10PLys14Ac complex, indicating that Lys9 acetylation decreases the binding affinity while Lys14 acetylation increase the binding affinity of the phosphorylated peptide. In this scenario, lysine acetylation may function as an ‘auxiliary modification’ that supports the relatively weak interaction of 14-3-3 ζ with H3Ser10P. Modified crystal structure (PDB ID: 2C1J) studies of the 14-3-3 ζ interaction with the H3 peptide containing phosphorylated Ser10 and acetylated Lys9 and

Lys14 suggested no major alterations in the interactions compared to in 14-3-3 ζ and H3 phosphopeptide complexes (Fig. 2). However, our in silico studies suggest that acetylation of Lys9 residue decreases the binding affinity. The alterations in hydrogen bonding and salt bridge formation due to introduction of acetyl groups resulting in charge neutralization may contribute to the decreased molecular interaction of 14-3-3 ζ with the H3 phosphoacetylated peptide.

The dual acetylation of Lys9 and Lys14 along with Ser10 phosphorylation allows one-step higher level of mechanism for controlling gene regulation. The dual modification will result in different combinations and provide different interaction sites for different interacting partners on the basis of charge or domain and may respond to distinct signaling pathways. In contrast, a previous study indicated no significant effect of dual H3Lys9/Lys14 acetylation on this interaction.⁶ Our data clearly indicates a modulation of the 14-3-3 ζ and histone H3 interaction by dual acetylation at Lys9 and Lys14 (Fig. 2B). This decreased binding affinity of 14-3-3 ζ with acetylation at Lys9 may result because the positive effect of single acetylation is neutralized by the dual acetylation or due to the close proximity of H3Ser10P with Lys9, which may affect the interaction of the phospho-domain of 14-3-3 ζ . The dual acetylation and phosphorylation may also modulate 14-3-3 ζ interactions with other regulatory proteins such as HATs, HDACs, and phosphatases, among others.

Docking of native MSK1 with histone H3 and its PTM modified structure

The dephosphorylation and phosphorylation of H3Ser10P in response to different stress-inducing agents such as oxidative stress and UV irradiation is a highly dynamic and reversible.^{34,35} MSK1 belongs to a family of protein kinases that contain two active domains in one polypeptide chain.³⁶ In the dual domain protein kinase MSK1, the N-terminal kinase has been shown to be phosphorylated by exogenous substrates, while the C-terminal kinase and the linker region act to regulate activity of the N-terminal kinase.¹⁹

The modeled structure of N-terminal domain of MSK1 (42–380 aa) was docked with native and acetylated histone H3 at Lys9 or Lys14 independently or together (Fig. 1B and Supplementary Fig. S3). The active site residues (Lys85, Ile88, Val89, Thr95,



Arg102, Gln122, and Leu127) of MSK1 were obtained from the literature.³⁶ The native histone H3 scored highest compared to acetylated Lys9 and Lys14 (Table 2). The bound state is stabilized by an intramolecular interaction. In native histone H3, the salt-bridge interaction was observed between Lys14 of H3 with Glu124 of MSK1, but when histone H3 was acetylated at either position, Lys9 forms a salt-bridge with Glu124 (Fig. 3 and Supplementary Fig. S4.2). Histone H3 acetylation at Lys14 favored an interaction with MSK1 with a Haddock score of -64.0 compared to a -47.4 score for Lys9 (Table 2). Haddock scores for the interaction of MSK1 with H3Ser10PLys9Ac and H3Ser10PLys14Ac clearly suggest that acetylation is not a prerequisite for phosphorylation of H3Ser10P by MSK1. The phosphoserine C α -C β bond is free to rotate. Thus, for an extended peptide that binds along the groove, two orientations are possible, differing by a two-fold rotation around the C α -C β bond. Therefore, the phosphorylated H3 tails may change their conformation to favor the interaction with 14-3-3 ζ . Since the amino-acid composition of histone H3 surrounding Ser10 does not match that of the high-affinity 14-3-3 ζ binding motifs, the postulated modulation of binding may only be relevant for a specific subset of 14-3-3 ζ -associated proteins with initial low-affinity binding to interact with other histone modifying enzymes such as HATs. Previous experimental results have suggested that the interaction of 14-3-3 ζ with the phosphorylated H3Ser10 favors the interaction of HATs responsible for acetylation of Lys14.¹² Collectively, the phosphorylation of Ser10 by MSK1 followed by low affinity interaction of 14-3-3 ζ and acetylation of Lys14 by HAT stabilizes the binding of 14-3-3 ζ to differentially modified H3 in interphase cells to interact with other proteins.

Docking of native MKP1 with histone H3 and its PTM structure

MKP1 is considered as a regulator which controls the access and recruitment of the transcriptional machinery to the promoter of targeted genes by dephosphorylating H3 on Ser10 after stimulation of endothelial cells by VEGF or thrombin.¹³ Previous studies by us and other groups have shown that in interphase cells, H3Ser10P is associated with acetylation of H3Lys9 and Lys14, which may favor gene transcription. This suggests that the interaction of MKP1 with H3Ser10P

will be in the neutralized positive charge surrounding due to Lys9 and Lys14 acetylation. However, the role of H3Lys9 and Lys14 acetylation in the dephosphorylation of H3Ser10 has been the subject of debate. These modifications are known to occur in response to the same stimuli, in the same tissue, and on the same histone tails.¹² In silico studies were carried out to understand the effect of neighboring acetylation on H3Lys9 and H3Lys14 on the dephosphorylation of H3Ser10P by MKP1.

The homology model structure of the C-terminal phosphatase domain of MKP1 (172–314 aa) was used for docking with the loop crystal structure of H3 peptide (1–21 aa) using the Haddock server (Fig. 1A and Supplementary Fig. S3). The active site amino acid, Cys258, and nearby residue important for the formation of the active site (His257, Cys258, Gln259, Ala260, Gly261, Ile262, Ser263, and Arg264) for MKP1 were obtained from UniProt ID: P28562. These residues were used for targeted docking native and modified histone H3 peptides. Haddock scores from all eight complexes suggested that the complex of MKP1 and H3Lys9AcSer10PLys14Ac showed the strongest binding compared to the complex containing the native histone H3 and MKP1 (Table 3, Supplementary Fig. S4.3 and Fig. 4). The Ser10 modification of histone H3 increased the binding affinity from -61.8 to -90.3 with MKP1. However, when either Lys9 or Lys14 acetylation was added with Ser10, the Haddock score increased from -90.3 to around -122 . The residues involved in the interaction with phosphorylated Ser10 are mostly from active site (Gln259, Ala260, Gly261, Ile262, Ser263, Arg264) of MKP1 observed in all the complexes. Based on this observation, acetylation at Lys9 or Lys14 may favor the interaction between Ser10 with MKP1. The modification of H3 at all the three locations (Lys9, Ser10, Lys14) further increased the hydrophobic interactions leading to form a stable and high-affinity interaction with MKP1 (Supplementary Fig. S4.3).

Our molecular modeling and previous studies suggest that the phosphorylation of H3Ser10 by MSK1 favors the recruitment of 14-3-3 ζ , which in turn recruits HATs and acetylates H3Lys9 and H3Lys14. Acetylation protects the phosphorylation at H3Ser10 from being removed by phosphatase and the acetylation of the H3 tail favors active chromatin organization, which facilitates gene transcription. The level of MKP1 is known



to increase in response to stress-inducing agents such as oxidative stress.¹⁵ Based on our *in silico* results, we hypothesize that the enhanced level of MKP1 may favor concentration-dependent binding and replacement of 14-3-3 ζ from H3Ser10PLys14Ac. The increased binding of MKP1 dephosphorylates H3Ser10P. The dephosphorylation of H3Ser10 favors deacetylation of H3Lys9/Lys14, which favors condensation of chromatin structure. Thus, these studies suggest that different proteins targeted to different PTMs can regulate chromatin structure and gene expression. However, our *in silico* findings can be validated by *in vitro* assays such as isothermal titration calorimetry and peptide pull-down coupled with mass spectroscopy to understand the interaction of proteins with no specific interacting domains for different types of posttranslational modifications.

Summary

The combinations of different modifications on histone tails alter the interaction of histone proteins with DNA and effector proteins. Histone H3Ser10 phosphorylation along with acetylation on neighboring lysine residues, Lys9 and Lys14, are highly dynamic modifications that regulate their interactions with other functional proteins in interphase cells to favor transcriptional activation. *In silico* studies revealed that phosphorylation is the prime event with interaction of 14-3-3 ζ followed by acetylation of Lys9 and Lys14 on the H3 tail. MKP1 dephosphorylates H3Ser10P in response to stress, but Lys9 and Lys14 acetylation favors the interaction of MKP1 with H3, whereas acetylation of Lys9 and Lys14 do not favor phosphorylation of H3Ser10 by MSK1. These data suggest that these dual modifications have implications for accessibility and further protein-protein interactions in response to different stimuli.

Author Contributions

Conceived and designed the experiments: SG, NG, AKS, AM. Analyzed the data: SG, NG, AKS, AM. Wrote the first draft of the manuscript: SG, NG. Contributed to the writing of the manuscript: AKS, AV. Agree with manuscript results and conclusions: SG, NG, AV, AKS, AM. Jointly developed the structure and arguments for the paper: SG, NG, AV. Made critical revisions and approved final version: SG, NG,

AKS. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

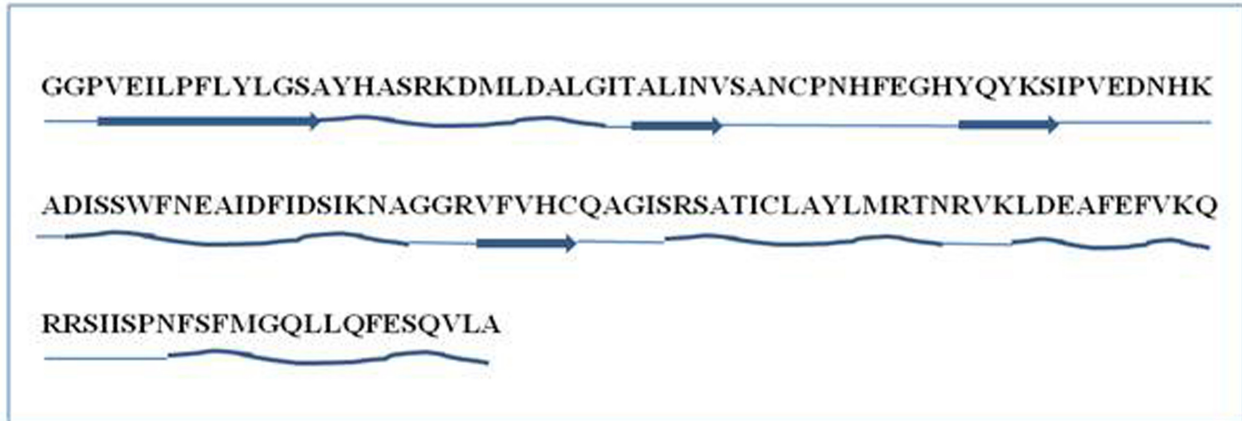
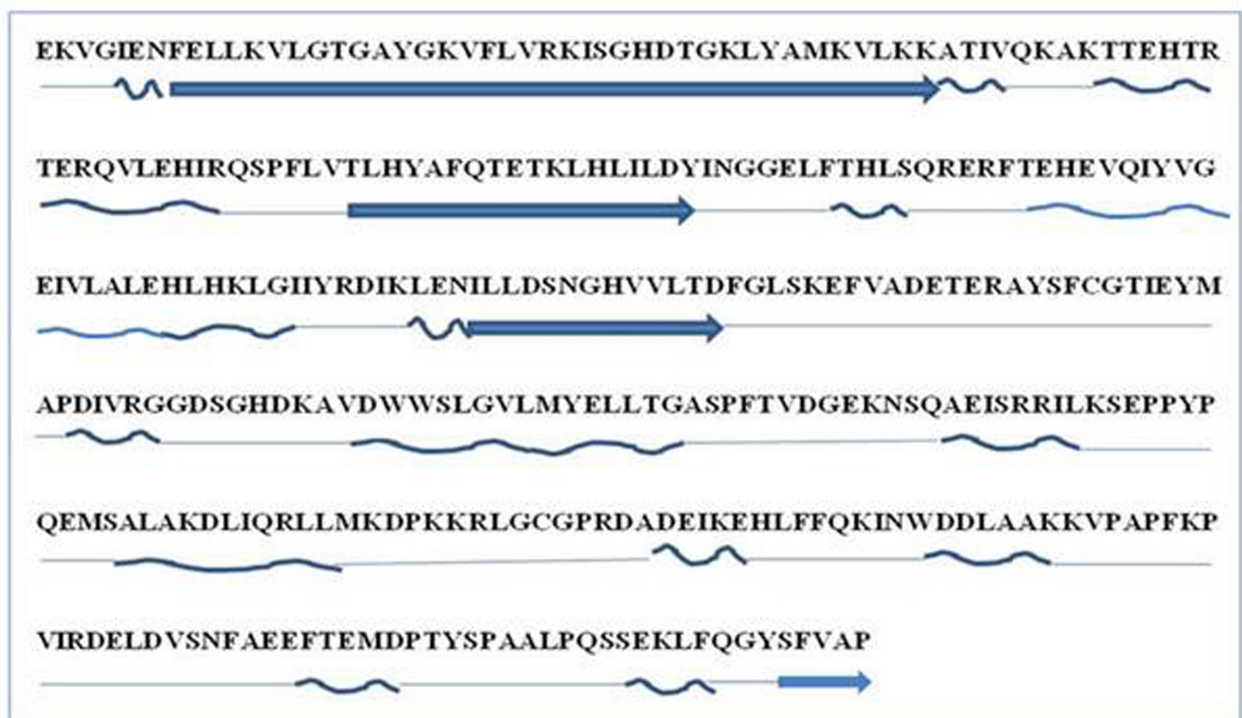
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Supplementary Figures

a. Secondary structure of C-terminal phosphatase domain of MKP1 (172-314)**b. Secondary structure of N-terminal kinase domain of MSK1 (42-380)****Key:**

Loop: —
Beta sheet: →
Alpha helix: ~

Figure S1. Line diagram for the secondary structures (A) C-terminal phosphatase domain of MKP1 (172–314 aa) and (B) N-terminal kinase domain of MSK1 (42–380 aa).

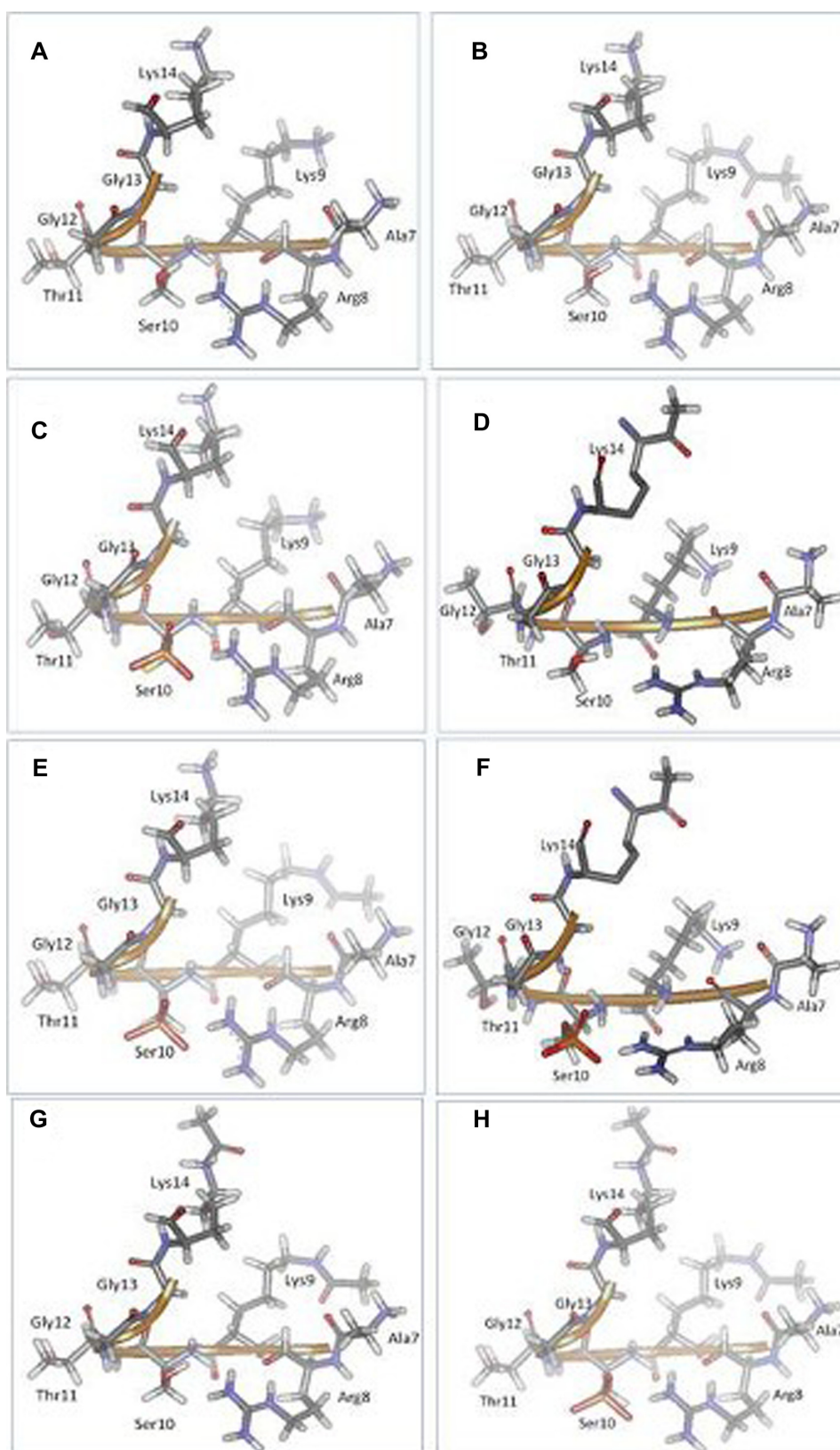


Figure S2. Histone H3 peptide from PDB: 2C1J was modified by phosphorylation of Ser10 and acetylation of Lys9 and Lys14. **(A)** Native histone H3, **(B)** H3Lys9Ac, **(C)** H3Ser10P, **(D)** H3Lys14Ac, **(E)** H3Lys9AcSer10P, **(F)** H3Ser10PLys14Ac, **(G)** H3Lys9AcLys14Ac, and **(H)** H3Lys9AcSer10PLys14Ac.

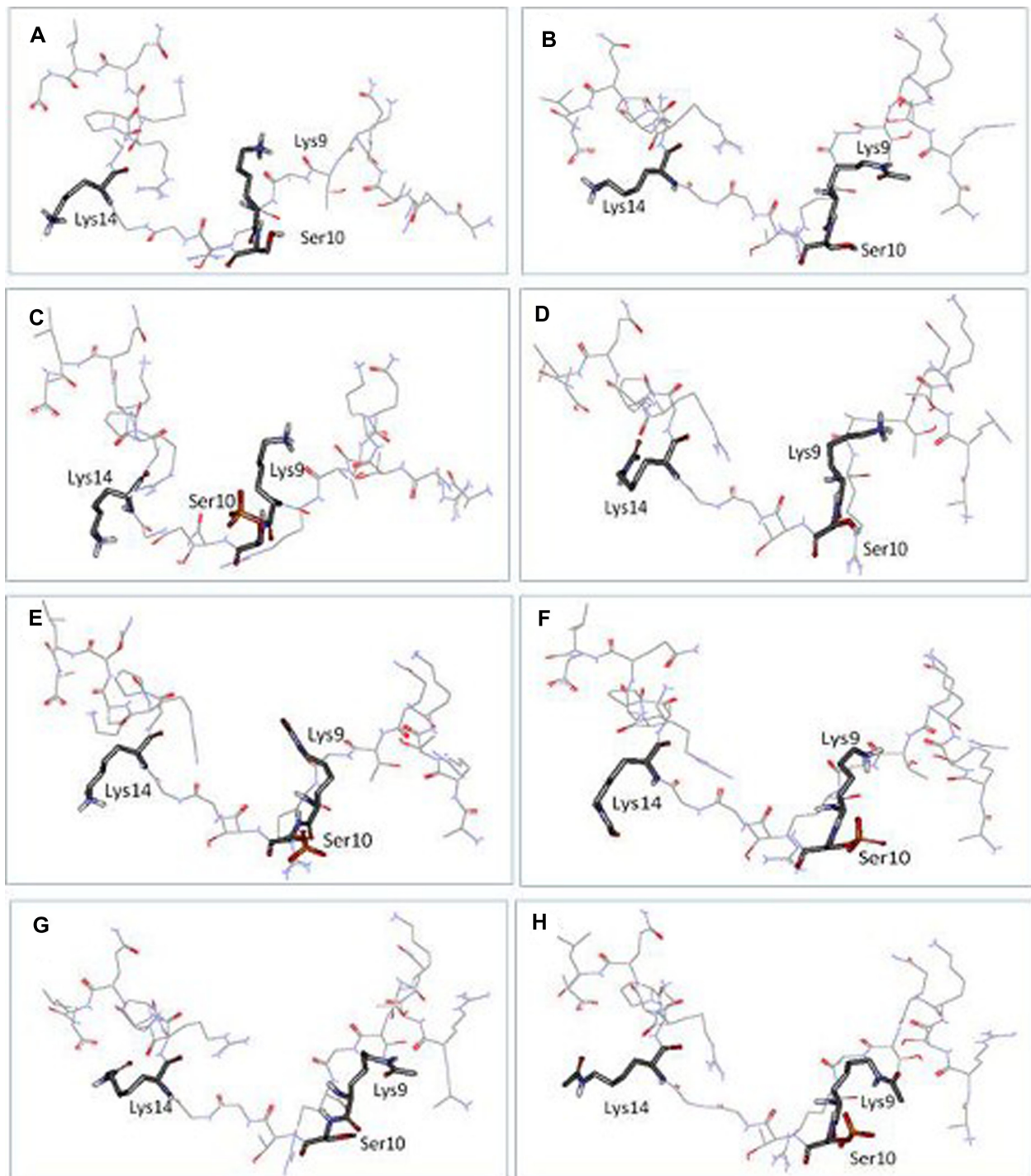


Figure S3. Full-length loop structure of histone H3 peptide from PDB: 1KX5 was modified by phosphorylation of Ser10 and acetylation of Lys9 and Lys14. (A) Native histone H3, (B) H3Lys9Ac, (C) H3Ser10P, (D) H3Lys14Ac, (E) H3Lys9AcSer10P, (F) H3Ser10PLys14Ac, (G) H3Lys9AcLys14Ac, and (H) H3Lys9AcSer10PLys14Ac.

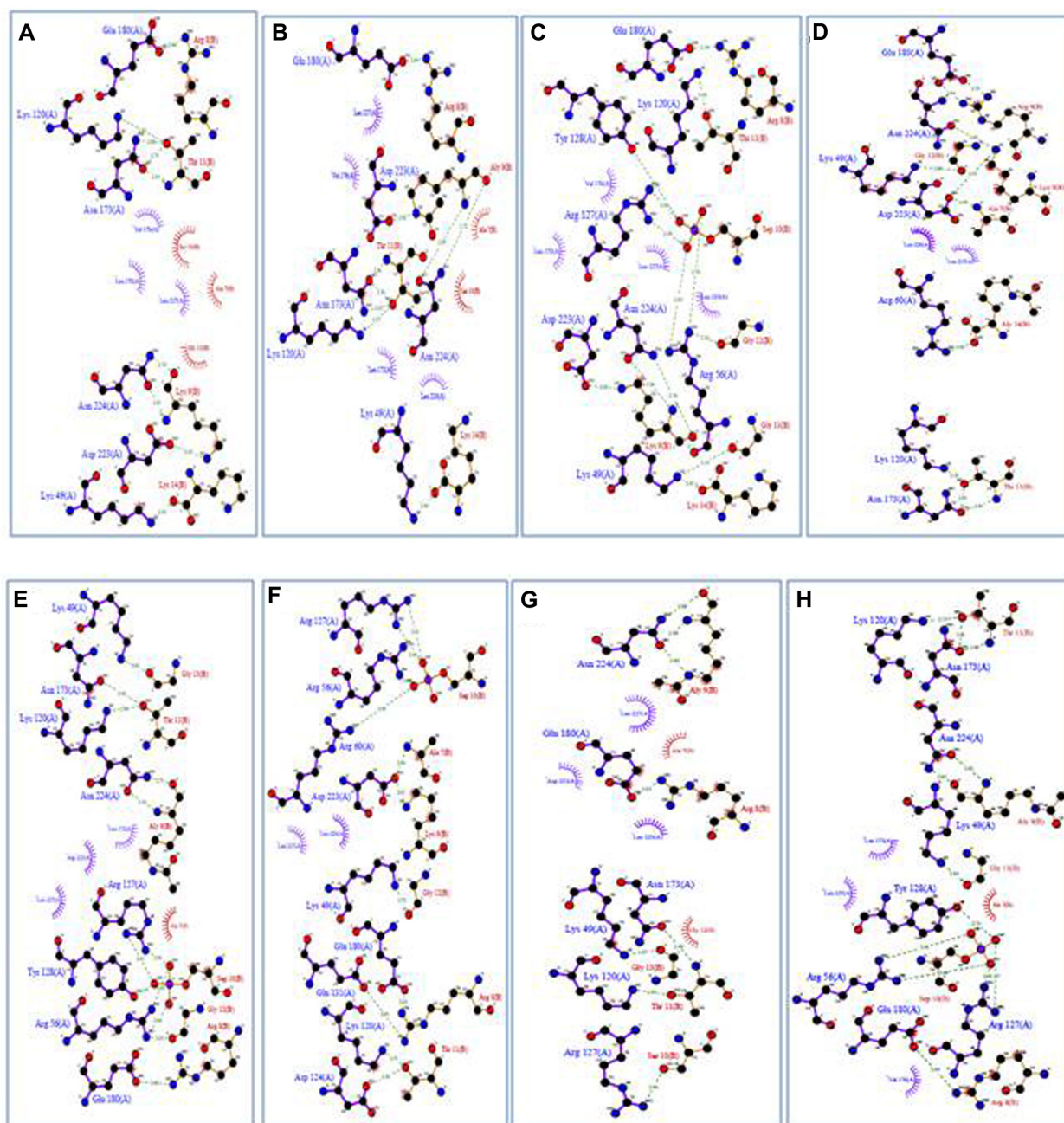


Figure S4.1. The Ligplot of the 14-3-3 ζ and histone H3 docked complexes to analyze hydrophobic interactions. (A) Native 14-3-3 ζ /H3, (B) 14-3-3 ζ /H3Lys9Ac, (C) 14-3-3 ζ /H3Ser10P, (D) 14-3-3 ζ /H3Lys14Ac, (E) 14-3-3 ζ /H3Lys9AcSer10P, (F) 14-3-3 ζ /H3Ser10PLys14Ac, (G) 14-3-3 ζ /H3Lys9AcLys14Ac, and (H) 14-3-3 ζ /H3Lys9AcSer10PLys14Ac.

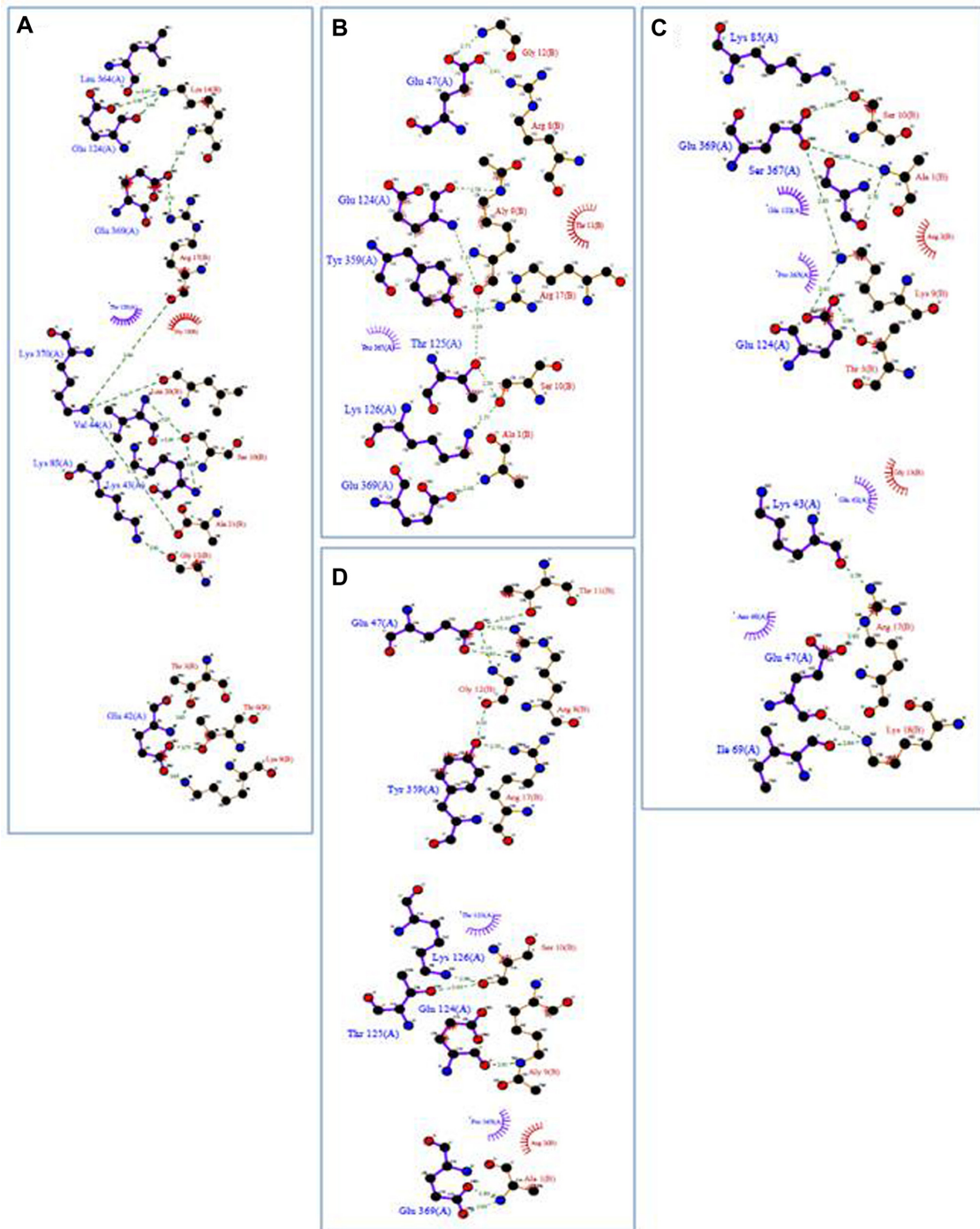


Figure S4.2. The Ligplot of the MSK1 and histone H3 docked complexes to analyze hydrophobic interactions. (A) Native MSK1/H3 (B) MSK1/H3Lys9Ac, (C) MSK1/H3Lys14Ac, and (D) MKP1/H3Lys9AcLys14Ac.

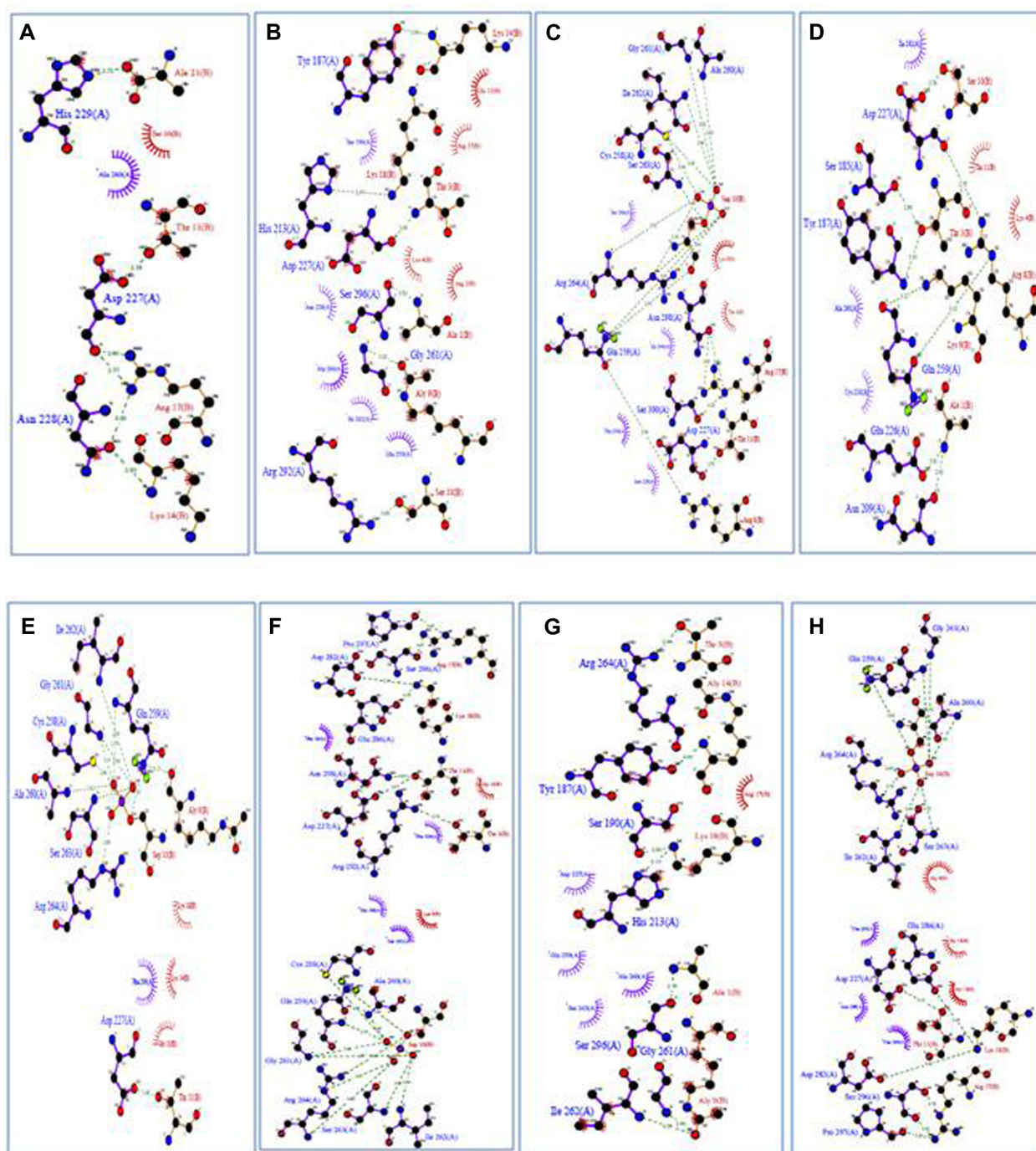


Figure S4.3. Ligplot of the MKP1 and histone H3 docked complexes to analyze hydrophobic interactions. (A) Native MKP1/H3 (B) MKP1/H3Lys9Ac, (C) MKP1/H3Ser10P, (D) MKP1/H3Lys14Ac, (E) MKP1/H3Lys9AcSer10P, (F) MKP1/H3Ser10PLys14Ac, (G) MKP1/H3Lys9AcLys14Ac, and (H) MKP1/H3Lys9AcSer10PLys14Ac.