http://doi.org/10.17952/38EPS.2026.P127

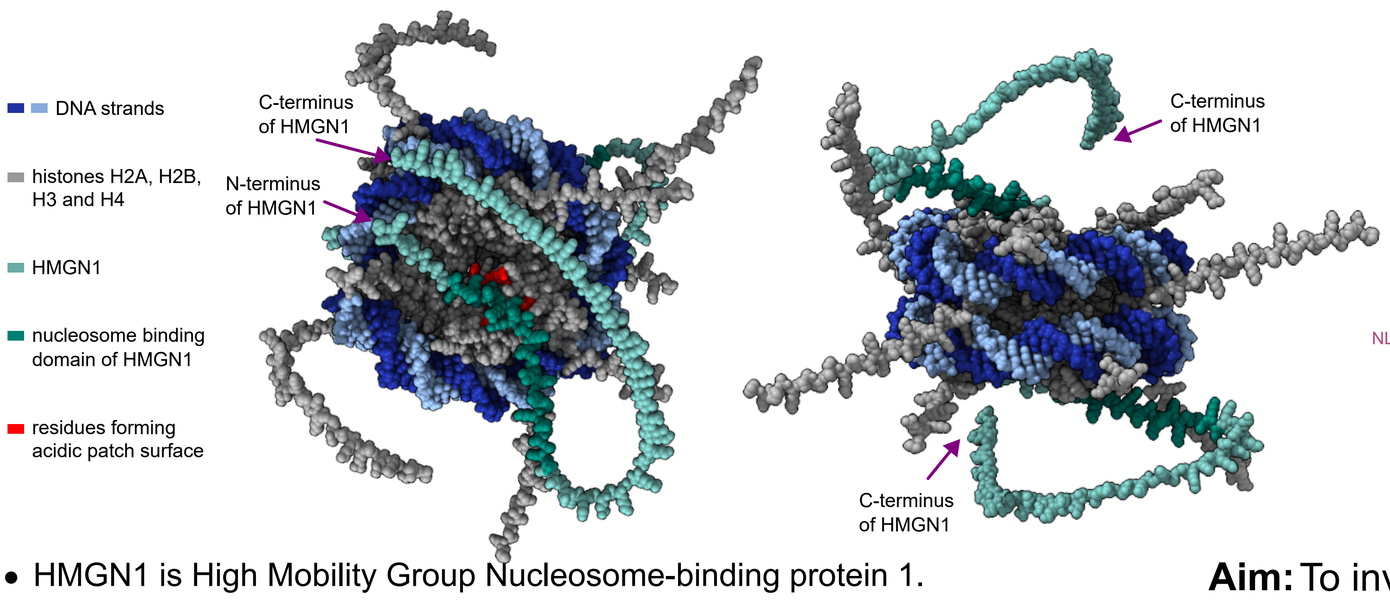
Lys acetylation and Ser phosphorylation crosstalk in the nucleosome binding domain of HMGN1

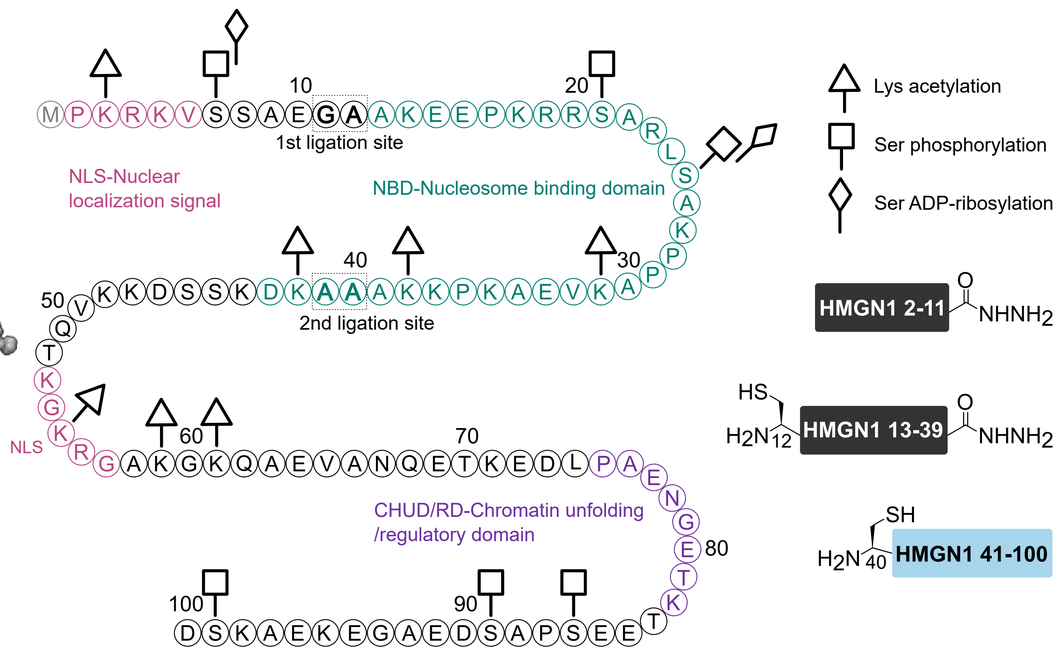
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E-mail: medine.soydan@tuwien.ac.at

Background

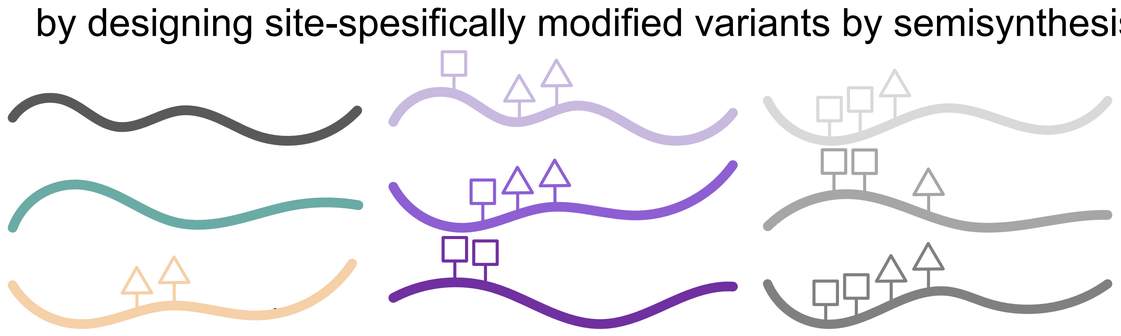
- Alphafold3 prediction of interactions between 2 molecules HMGN1 and nucleosome





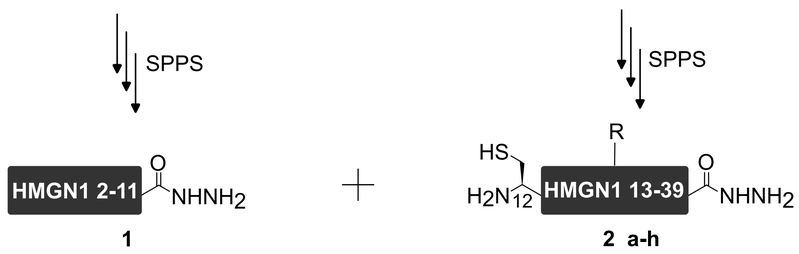
- HMGN1 is High Mobility Group Nucleosome-binding protein 1.
- HMGN1 is non-histone nucleosome binding protein interacting through the acidic patch surface.
- Acidic patch surface is Glu rich surface between H2A-H2B.
- HMGN1 is intrinsically disordered.
- HMGN1 undergoes extensive post-translational modifications (PTMs).

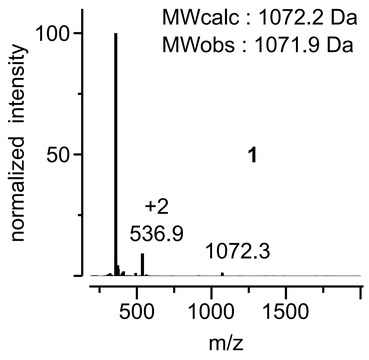
Aim: To investigate crosstalk between Lys acetylation and Ser phosphorylation in NBD of HMGN1 in nucleosome binding by designing site-spesifically modified variants by semisynthesis.



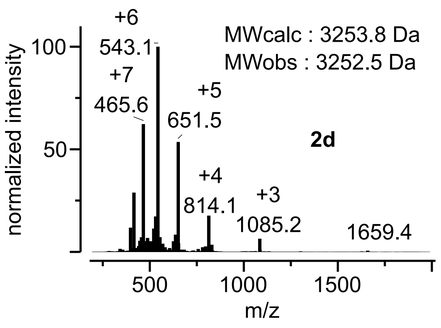
Synthesis

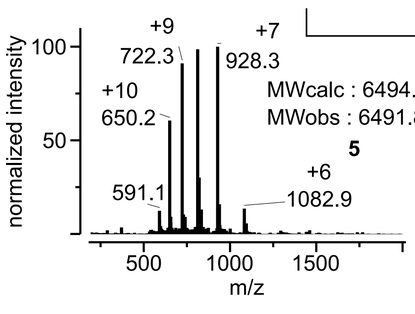
- 3 steps semisynthesis in N-to-C direction
- Ala to Cys mutation at Ala12 and Ala40



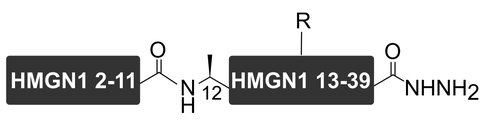


2a R=unmodified
2b R=acK31acK38
2c R=pS21acK31acK38
2d R=pS25acK31acK38
2e R=pS21pS25
2f R=pS21pS25acK31
2g R=pS21pS25acK38
2h R=pS21pS25acK31acK38



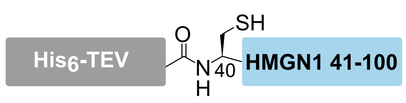


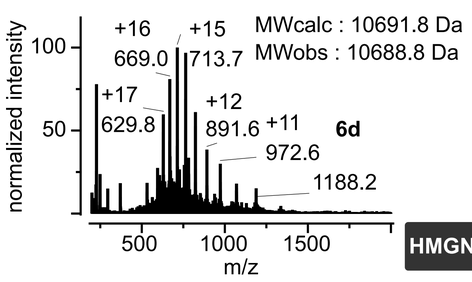
1i. Oxidation
1ii. Thiolysis
1iii. Ligation
1iv. Desulfurization



Ni-NTA affinity chromatography

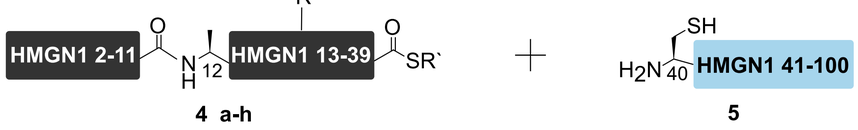
Recombinant expression in *E. coli*

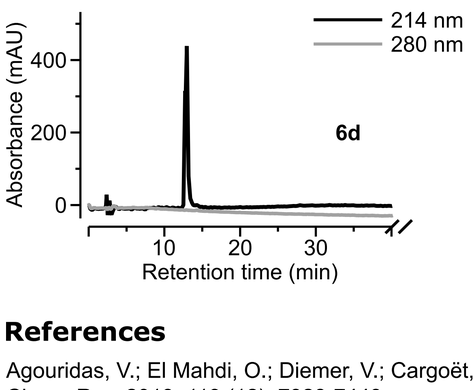




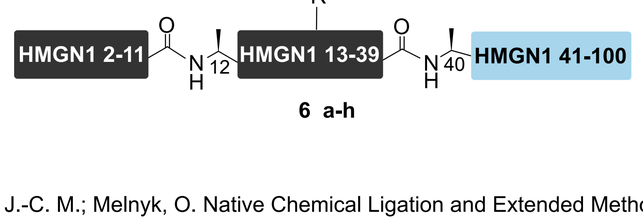
2i. Oxidation
2ii. Thiolysis

protease cleavage



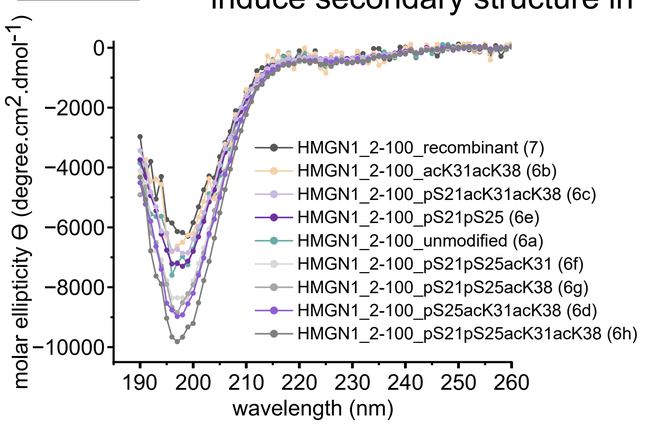


3i. Ligation
3ii. Desulfurization



Analysis

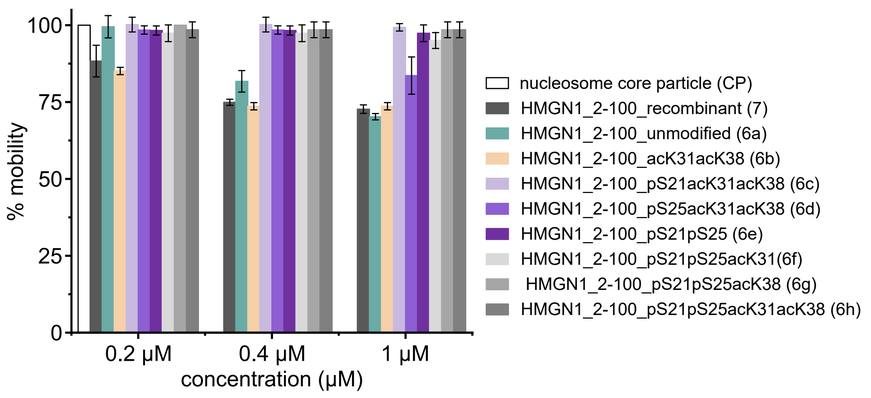
- Circular dichroism shows that modifications did not induce secondary structure in whole protein.



- All variants are random coil.
- Phosphorylation of Ser21 and/or Ser25 abolishes nucleosome binding, regardless of Lys31 and/or Lys38 acetylation.
- >1.2× TBE buffer (1× TBE: 89 mM Tris, 89 mM borate, 1 mM EDTA, pH 8.4) → cooperative binding (i.e., 2 molecules HMGN1/nucleosome) → 2× TBE buffer was used

A representative native gel

- 3 experimental replicates of EMSA gels were performed.
- 20 R R S A R L S A — core conserved sequence of NBD in HMGN family
- Partial recovery of binding in 6d with 5 equiv. implies the critical role of Ser21 in nucleosome binding.
- As Ser phosphorylation has a dominating effect in abolishing the binding affinity, a crosstalk effect between Ser phosphorylation and Lys acetylation was not observed



References

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Postnikov, Y. V.; Bustin, M. Functional Interplay between Histone H1 and Hmg Proteins in Chromatin. Biochim. Biophys. Acta Gene Regul. Mech. 2016, 1859 (3), 462-467.
Niederacher, G.; Urwin, D.; Dijkwel, Y.; Tremethick, D. J.; Rosengren, K. J.; Becker, C. F. W.; Conibear, A. C. Site-Specific Modification and Segmental Isotope Labelling of Hmgn1 Reveals Long-Range Conformational Perturbations Caused by Posttranslational Modifications. RSC Chem. Biol. 2021, 2 (2), 537-550.

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