Elucidating the Driving Forces Behind UHRF1 TTD-PHD Binding to the Histone 3 Tail

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Background and Motivations

- Histones are modified with covalent marks after translation
- Trimethyllysine (Kme3) is a post-translational modification (PTM) often recognized through an aromatic cage configuration
- Cation-π interactions typically drive binding
- UHRF1 TTD-PHD unexpectedly binds *tert*-butyl norleucine (tBuNle) with equal affinity

Goal to identify the forces driving binding in this system

Workflow

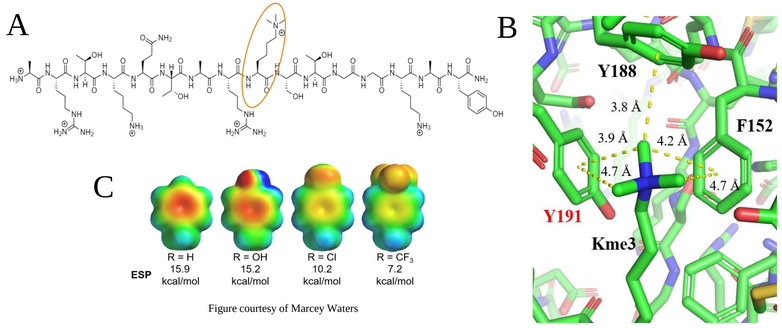

Figure courtesy of Marcey Waters

Figure 2. (A) H3K9me3(1-15)-Y structure (B) UHRF1 TTD-PHD aromatic cage interacting with H3K9me3 (C) Electrostatic potential map of the aromatic portion of *para*-phenylalanine derivatives

1. Synthesize H3K9me3(1-15)-Y and H3K9tBuNle(1-15)-Y peptides
2. Tune the Y191 aromatic cage residue with functional groups varying in electrostatic potential using Genetic Code Expansion (GCE)
3. Use Isothermal Titration Calorimetry (ITC) to determine the thermodynamic parameters of binding

PTM Recognition and UHRF1 Structure

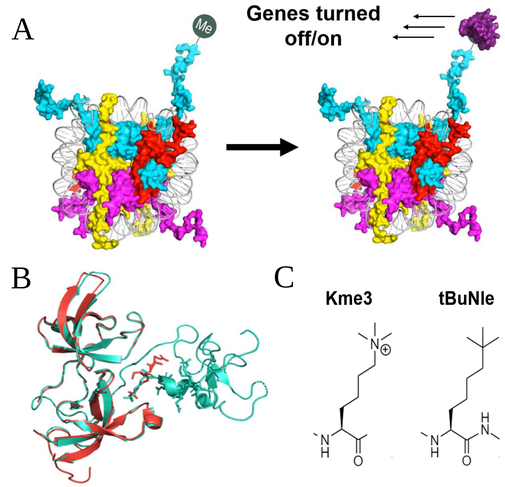

Figure 1. (A) Reader protein interacting with nucleosome (B) UHRF1 TTD-PHD interacting with H3K9me3 (C) Structures of Kme3 and tBuNle

Thermodynamics of Binding and Free Energy Correlations

- All mutants exhibit minimal perturbation in binding
- Binding to Kme3 is more enthalpically driven while binding to tBuNle is more entropically driven
- No correlations between free energy of binding and ESP, logP, or polarizability of the aromatic cage for Kme3 or tBuNle binding

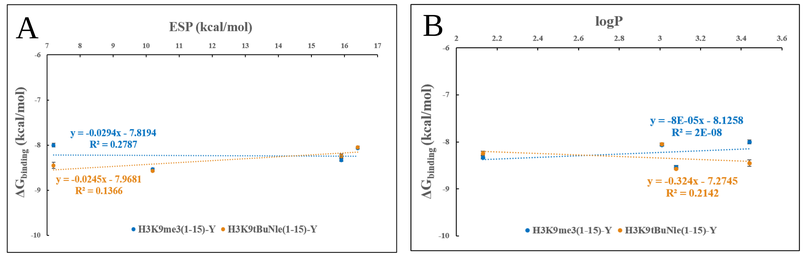

Figure 3. (A) ESP vs. free energy of binding to H3K9me3(1-15)-Y and H3K9tBuNle(1-15)-Y (B). logP vs. free energy of binding to H3K9me3(1-15)-Y and H3K9tBuNle(1-15)-Y

Conclusion

- The aromatic cage within the TTD domain does not significantly influence binding to the H3 tail in the UHRF1 dual-domain system
- Favorable acidic contacts between various PHD domain residues and arginine 2 of histone 3 are likely responsible for binding

Future Directions

- Structurally alter PHD domain acidic contact points using similar mutagenesis studies to conclusively determine whether the PHD domain drives binding
- Perform similar electrostatic tunability studies on the single-domain UHRF1 TTD system to determine whether the aromatic cage is intrinsically non-tunable or whether the addition of the PHD domain is responsible for its non-tunability.

References

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12. Dr. Marcey Waters
13. Dr. Stephen Baril
14. Noah Schomburg

Acknowledgments

This work was funded by National Institute of General Medical Sciences of the National Institutes of Health under award numbers R01 GM118499 and R35 GM145227 to M.W.