MOLECULAR SIMULATION STUDIES OF PROTEIN INTERACTIONS INVOLVED IN AGING: INVESTIGATING THE RECRUITMENT MECHANISM OF PRC2 AND DNMT3A

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BACKGROUND

- The deterioration of the epigenome over time is currently understood as one of the primary causes of aging [1].
- The epigenetic pattern within a cell is maintained by epigenetic modifier proteins. But the mechanism through which these proteins are able to be specifically targeted to certain sites throughout the genome remains unclear [2].
- Two epigenetic modifier proteins, DNA Methyltransferase 3A (DNMT3A) and the Polycomb Repressive Complex 2 (PRC2), have been experimentally determined to recruit each other throughout the genome to catalyze epigenetic modification at specific sites [3], but the exact mechanism of this interaction remains unclear.
- Molecular dynamics simulations can be used as a method of studying protein-to-protein interactions through computationally simulating how two proteins would interact with each other.

PRIMARY OBJECTIVE

- The primary objective of this project was to investigate the recruitment interaction between DNMT3A and PRC2 using docking and molecular dynamics simulations.
- Through pursuing this objective it was hoped a broader understanding of epigenetic modifier protein recruitment could be gained, and conversely how the decay of these proteins contributes to aging.

METHODS

- Computational models of DNMT3A and PRC2 were created from the PDB files 5HYN (PRC2) and 4U7T (DNMT3A) by filling in missing residues and adding hydrogen atoms to the structures.
- Docked models of DNMT3A and PRC2 were created using the program HDOCK.
- The docked structures were examined, and it was noted the 3 highest rated models were docked at essentially the same locations, but at different orientations (see Figure 1).
- The 3 highest rated docked structures had a molecular dynamics simulation run with 1000 steps of minimization, 100 ps of NPT, and 20 ns of NVT.

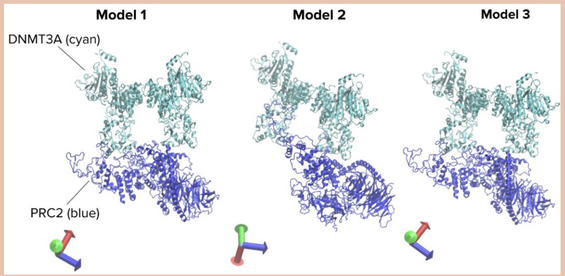

Figure 1. This image presents the 3 docked models of DNMT3A and PRC2 that underwent simulation.

RESULTS

- Based on a visual examination of the simulation results, it appeared that the SANT I & II domains of PRC2 were crawling up the ADD domain of DNMT3A. The three different docked conformations appeared to all demonstrate this similar behavior.

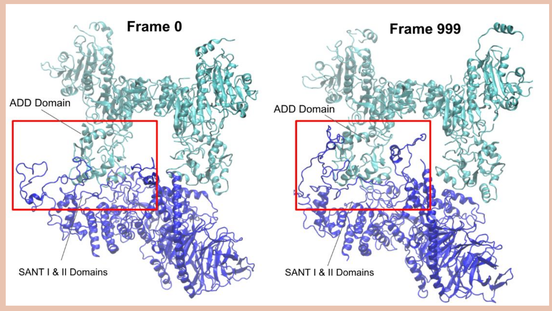

Figure 2. This image presents the first and last frame of the simulation of the interaction between DNMT3A (cyan) and PRC2 (dark blue) for model 1. Progressing from frame 0 to frame 999, the SANT I & II domains of PRC2 can be seen to extensively interact with the ADD domain of DNMT3A.

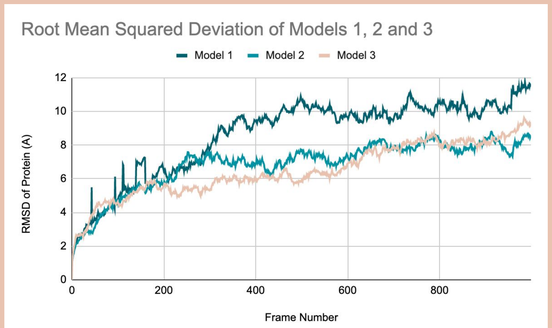

Figure 3. Graph demonstrating the RMSD scores over time for models 1, 2 and 3. All of the models' RMSD scores generally even out over the course of the simulation, suggesting the protein complexes are stable.

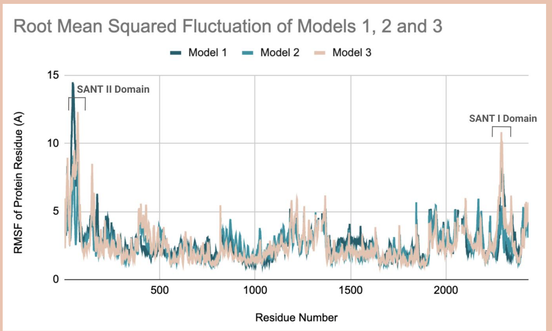

Figure 4. Graph demonstrating the average RMSF scores over time for models 1, 2 and 3. Large spikes can be seen for the SANT I and SANT II domains of PRC2, which suggests the configuration of these domains changes extensively over the course of the simulation.

EVIDENCE OF THE ADD & SANT I DOMAINS INVOLVEMENT IN THE RECRUITMENT MECHANISM

- The simulation results suggesting that the ADD domain and SANT I domain are involved in the recruitment interaction between DNMT3A and PRC2 are supported by the original experimental data, which reported the recruitment interaction involved amino acids 1-340 in the EZH2 subunit of PRC2 and amino acids 490-582 in DNMT3A [3] (see Figure 5). These amino acid segments include the SANT I domain in PRC2 and the ADD domain in DNMT3A.
- Additionally, within the amino acid segment reported to be involved in the recruitment interaction, the SANT I domain is one of the only physically accessible portions of PRC2 when it is bound to a histone [5] (see Figure 6).
- Finally, a previous study had reported that the SANT I domain of PRC2 was responsible for recruiting MYC, an epigenetic modifier protein similar to DNMT3A, to specific areas of the genome [6].

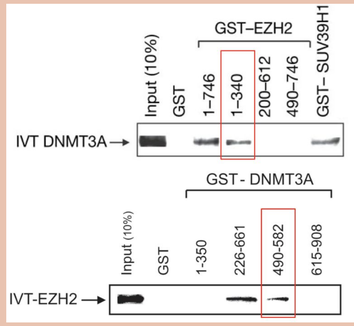

Figure 5. This image comes from the original paper first reporting experimental evidence that PRC2 recruits DNMT3A to specific locations throughout the genome [3]. This image presents the results of a GST pull down assay from this paper, which provided information on what segments of DNMT3A and the PRC2 subunit EZH2 are specifically involved in the recruitment interaction. The red boxes highlight the areas shown to be most relevant to this interaction.

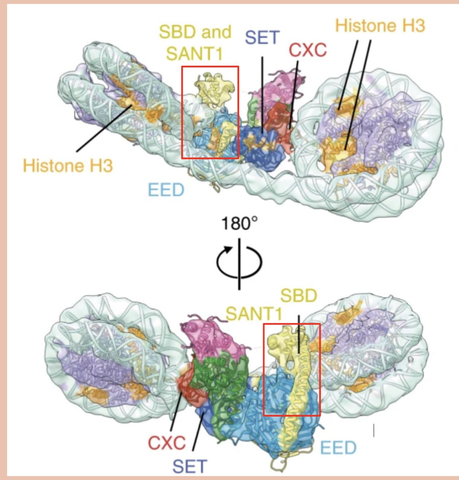

Figure 6. This image from [5] demonstrates the conformations PRC2 takes when bound to a histone. The red box roughly demonstrates the location of amino acids 1-340 in the EZH2 subunit of PRC2, the majority of which are buried within PRC2. As can be seen, the SANT I domain is one of the only externally exposed parts of this segment of amino acids, further suggesting its prominent role in PRC2's recruitment mechanism for DNMT3A.

DISCUSSION

- I hypothesize that the SANT I domain of PRC2 and the ADD domain of DNMT3A play a prominent role in the recruitment mechanism of PRC2 and DNMT3A, based on the simulation results generated so far, in addition to the literature evidence.
- The next step in assessing the validity of this hypothesis would be to conduct large scale molecular dynamics simulations with the docked model of the ADD domain of DNMT3A and the SANT I domain of PRC2 shown in Figure 7.
- Through an analysis of the simulation data generated, further conclusions could be drawn on how the SANT I and ADD domain interact with each other, and how they are involved in the recruitment mechanism between DNMT3A and PRC2.

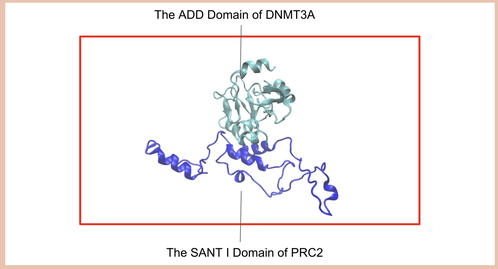

Figure 7. This image presents the highest rated docked model of the ADD domain of DNMT3A and the SANT I domain of PRC2. The red box demonstrates which area of the whole complex this structure originates from in Figure 2.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Sinclair, D. A. (2023). Loss of epigenetic information as a cause of mammalian aging. *Cell*, *186*(2), 305–326.e27. https://doi.org/10.1016/j.cell.2022.12.027

[2] Laisné M, Gupta N, Kirsh O, Pradhan S, Defossez PA. Mechanisms of DNA Methyltransferase Recruitment in Mammals. Genes (Basel). 2018 Dec 10;9(12):617. doi: 10.3390/genes9120617. PMID: 30544749; PMCID: PMC6316769.

[3] Viré, E., Brenner, C., Deplus, R. *et al.* The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* 439, 871–874 (2006). https://doi.org/10.1038/nature04431

[4] Johnson AA, Akman K, Calimport SR, Wuttke D, Stolzing A, de Magalhães JP. The role of DNA methylation in aging, rejuvenation, and age-related disease. Rejuvenation Res. 2012 Oct;15(5):483-94. doi: 10.1089/rej.2012.1324. PMID: 23098078; PMCID: PMC3482848.

[5] Poepsel, S., Kasinath, V. & Nogales, E. Cryo-EM structures of PRC2 simultaneously engaged with two functionally distinct nucleosomes. *Nat Struct Mol Biol* 25, 154–162 (2018). https://doi.org/10.1038/s41594-018-0023-y

[6] Wang, J., Yu, X., Gong, W., Liu, X., Park, K. S., Ma, A., Tsai, Y. H., Shen, Y., Onikubo, T., Pi, W. C., Allison, D. F., Liu, J., Chen, W. Y., Cai, L., Roeder, R. G., Jin, J., & Wang, G. G. (2022). EZH2 noncanonically binds cMyc and p300 through a cryptic transactivation domain to mediate gene activation and promote oncogenesis. *Nature cell biology*, *24*(3), 384–399. https://doi.org/10.1038/s41556-022-00850-x