Betty A. and Donald J. Baumann Family Scholarship Fund Application Form

1. Name and NetID

Ivy Williams (ilw30763)

2. Chemistry faculty research director

Dr. Lynne Dieckman

3. Proposal title

Kinetic Characterization of the Interaction between PCNA and CAF-1 using Surface Plasmon Resonance.

4. Proposal description. Please limit the proposal to about 500 words and include figures as appropriate. Your proposal should briefly outline the overall project and its goal(s). If you have previous results related to your proposed project, concisely summarize those results and describe what you expect to accomplish during the time frame of the scholarship.

Genomic DNA in eukaryotes must be tightly packed to fit inside the cell nucleus. To properly organize DNA, it is coiled around eight histone proteins to form nucleosomes, which are further condensed to form chromatin[1]. Depending how tightly the nucleosomes are packed dictates whether a gene will be expressed or silenced. The packaging of nucleosomes in regions of silenced genes requires the coordinated action of two key proteins: proliferating cell nuclear antigen (PCNA) and chromatin assembly factor-1 (CAF-1)[2-3]. PCNA is a homotrimeric sliding clamp protein that acts as a scaffold to stabilize proteins involved in DNA templated processes. CAF-1 is a heterotrimeric histone chaperone protein. PCNA recruits CAF-1 to the replication fork to deposit histones on newly synthesized DNA to repackage the DNA into nucleosomes[4-5](Figure 1). If PCNA and CAF-1 are unable to interact, nucleosome assembly and gene silencing is disrupted, resulting in genome instability and disease[6]. The precise mechanism governing the PCNA-CAF-1 interaction remains unclear. Our lab is focuses on studying the structural, thermodynamic, and kinetic basis of the PCNA-CAF-1 interaction. My specific role on the research team is to determine the binding kinetics of CAF-1 and PCNA using surface plasmon resonance (SPR).

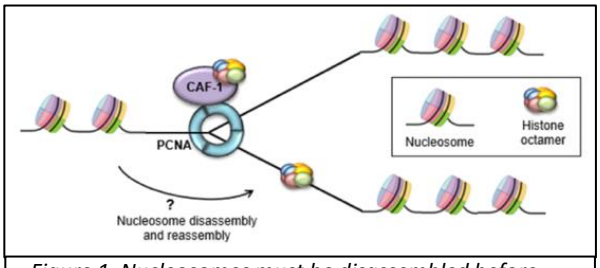

Figure 1. Nucleosomes must be disassembled before replication and reassembled after replication. This process requires the CAF-1-PCNA interaction.

SPR is a sensitive optical technique that measures refractive index changes on a gold surface to detect biomolecular interactions in real-time. In this process, one protein is immobilized on a sensor chip while another protein is flowed over it, generating association and dissociation curves that enable the calculation of kinetic parameters (Figure 2). Proteins that bind to PCNA do so via a conserved PCNA interacting peptide (PIP) motif[7]. The PIP motif binds in the hydrophobic pocket on the front face of PCNA (Figure 3). Our lab has identified two PIP motifs in the largest subunit of yeast CAF-1, Cac1. The canonical motif in the center of the Cac1 subunit is called PIP2, and the N-terminal PIP motif identified thorough sequencing analysis is called PIP1 (Figure 4).

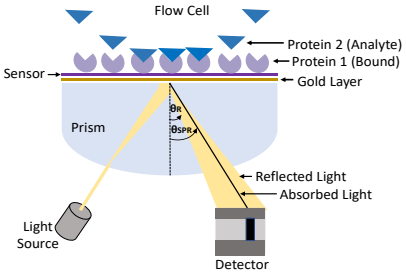

Figure 2. SPR sensor mechanism. Protein 1 is immobilized on a gold sensor chip, and protein 2 is flowed over the chip. Light is shot at the sensor chip, and the change in the SPR response angle is measured.

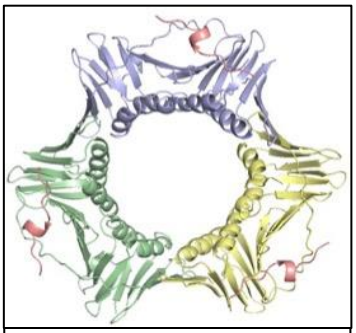

Figure 3. Crystal structure of the CAF-1 PIP motif (pink) binding to PCNA. Each subunit of PCNA is shown in a different color.

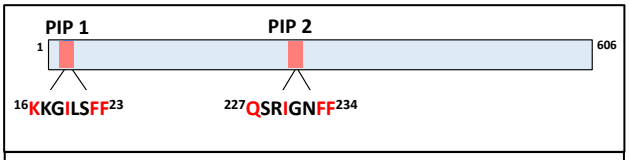

Figure 4. Sequences and locations of the two PIP motifs in Cac1. The conserved PIP residues are highlighted in red.

I have determined the kinetics of binding of each individual PIP motifs to PCNA (Figure 5). The data in Figure 5A and 5B was recently published in the Journal of Molecular Biology, which I am a coauthor of[8]. The affinity of the PIP2 motif of CAF-1 binding to PCNA was determined to be 4 µM, and the affinity of the PIP1 motif of CAF-1 binding to PCNA was determined to be 120 µM. The fact that the two PIP motifs of CAF-1 have drastically different affinities for PCNA suggests these motifs may have differing

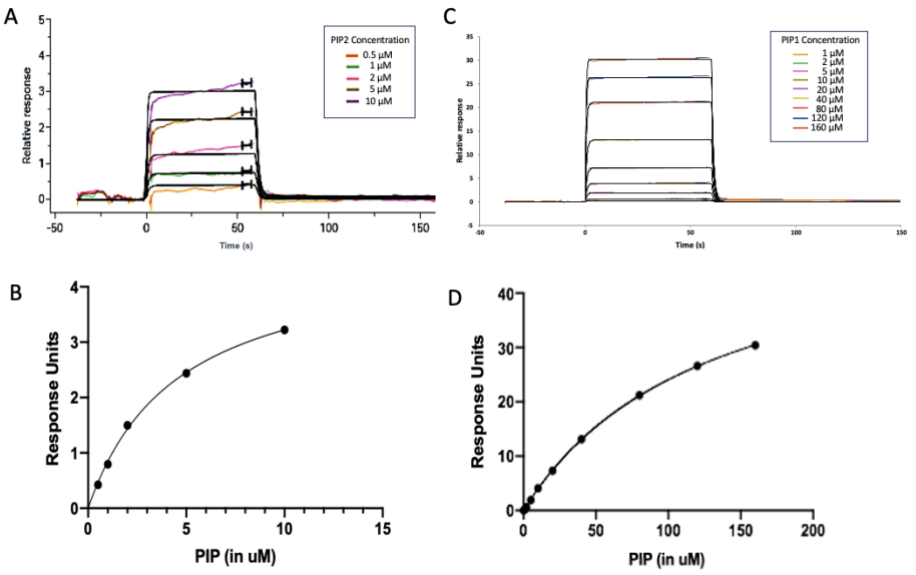

Figure 5. (A) Kinetics of the PIP2 motif of CAF-1 binding to immobilized PCNA fit to a 2-step binding equation. A 2-step mechanism suggests a quick association followed by a slower confirmational change upon binding (k_1=1.37 × 10^5 ± 2.4 × 10^3 $M^{-1}s^{-1}$, k_{-1}= 7.43 × 10^{-1} ± 1.3 × 10^{-2} s^{-1}, k_2 = 6.18 × 10^{-4} ± 2.6 × 10^{-5} s^{-1}, k_{-2} = 1.67 × 10^{-3} ± 3.2 × $10^{-4}s^{-1}$).(B) Equilibrium responses for each PIP2 concentration were plotted to obtain steady state affinity (K_d = 4.0 ± 0.6 µM). (C) Kinetics of the PIP1 motif of CAF-1 binding to immobilized PCNA fit to a 1:1 binding equation. The on and off rates of binding are very fast (k_{on} = 9.35 x 10^3 $M^{-1}s^{-1}$, k_{off} = 1.13 s^{-1}) (D) Equilibrium responses for each PIP1 concentration were plotted to obtain steady state affinity (K_d =120µM ± 6 µM).

functions in the cell. We hypothesize that both motifs are necessary for a high affinity interaction with PCNA.

In the next semester, I plan to conduct SPR studies to determine the kinetics of binding between the full-length Cac1 protein and PCNA to determine the mechanism of binding when both PIP motifs are present. This will require the optimization of an alternate sensor chip surface chemistry to study the Cac1-PCNA interaction, completion of SPR experiments, and SPR data analysis. I hypothesize the full Cac1 subunit will bind even tighter than either of the PIP motifs alone. Coupling this kinetic data with structural and thermodynamics studies of the PCNA-CAF-1 interaction obtained in our lab will provide a comprehensive understanding of how CAF-1 and PCNA interact to regulate nucleosome assembly and maintain genomic stability.

References

[1] Grunstein, M., Hecht, A., Fisher-Adams, G., Wan, J., Mann, R. K., Strahl-Bolsinger, S., Laroche, T., Gasser, S. (1995). The regulation of euchromatin and heterochromatin by histones in yeast. *Journal of Cell Science*. 19, 29-36. https://doi.org/10.1242/jcs.1995.supplement_19.4

[2] Zhang, K., Gao, Y., Li, J., Burgess, R., Han, J., Liang, H., Zhang, Z., and Liu, Y. (2016). A DNA binding winged helix domain in CAF-1 functions with PCNA to stabilize CAF-1 at replication forks. *Nucleic acids research, 44*(11), 5083-5094. https://doi.org/10.1093/nar/gkw106

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[4] Hoek, M., Stillman, B. (2003). Chromatin assembly factor 1 is essential and couples chromatin assembly to DNA replication in vivo., *Proceedings of the National Academy of Sciences of the United States of America, 100*(21), 12183-12188. https://doi.org/10.1073/pnas.1635158100

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5. **Presentation of research results (past and future conferences, publications, seminars, etc.)**

Publications

Orndorff, K., Veltri, E., Hoitsma, N., **Williams, I.**, Hall, I., Jaworski, G., Majeres, G., Kallepalli, S., Vito, A., Struble, L., Borgstahl, G., Dieckman, L. M. (2024). *Structural Basis for the Interaction Between Yeast Chromatin Assembly Factor 1 and Proliferating Cell Nuclear Antigen*.: Journal of Molecular Biology.

Regional and National Presentations

Williams, I., Carlson, C., and Dieckman L., *Kinetic Studies of Proteins Involved in Gene Silencing Using Surface Plasmon Resonance.* Poster Presentation at ACS Midwest Regional Meeting, Omaha, NE, October 15, 2024.

Williams, I., Carlson, C., and Dieckman, L., *Kinetic Studies of Proteins Involved in Gene Silencing Using Surface Plasmon Resonance.* Poster Presentation at the 10th Structural and Biophysics Worshop, Omaha, NE, August 1, 2024.

Williams, I., and Dieckman, L., *Kinetic Studies of Proteins Involved in Gene Silencing Using Surface Plasmon Resonance.* Poster Presentation at the Heartland Undergraduate Biochemistry Forum, Kansas City, KS, November 11, 2023.

Statewide and Local Presentations

Williams, I., and Dieckman, L., *Kinetic Studies of Proteins Involved in Gene Silencing.*
Oral Presentation at Nebraska Academy of Sciences Annual Spring Meeting, Lincoln, NE, April 19, 2024.

Williams, I., and Dieckman, L., *Kinetic Studies of Proteins Involved in Gene Silencing.*
Oral Presentation at Creighton University Research Week, Omaha, NE, March 26, 2024.
***Awarded best undergraduate oral presentation in biomedical sciences**

Williams, I., and Dieckman, L., *Kinetics of Proteins Involved in Nucleosome Assembly Using Surface Plasmon Resonance.* Poster Presentation at Omaha ACS Mid-summer Presentation Session, Omaha, NE, July 6, 2023.

Williams, I., Mishra, S., Lee, A., Bushong, M., and C. Fassbinder-Orth. *Expression of OBP-14 Concentration in Different Age and Caste of Apis Mellifera in Correlation to Odorant Functional Coding*. Poster Presentation at Creighton University Research Week, Omaha, NE, April 19-20, 2022.

6. **Post-graduate plans (job market, graduate school, medical school, etc.)**

I plan to attend graduate school in pursuit of a PhD in Biochemistry

7. **Number of semesters involved in research, including current semester (summers count as two semesters)**

10 Semesters

8. **Anticipated graduation date**

May 2025