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

1. **Name and NetID**
   Molly Dolan
   Mmd86774

2. **Chemistry faculty research director**
   Dr. Lynne Dieckman

3. **Proposal title**
   Using Small Angle X-Ray Scattering and X-Ray Crystallography to Investigate Structural Changes in Proteins Involved in Gene Silencing

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.**

In eukaryotic cells, DNA is packaged and organized into chromatin to fit into the cell's nucleus and to preserve genomic integrity. The basic unit of chromatin is the nucleosome, which is composed of approximately 147 base pairs of newly synthesized DNA wrapped around a histone octamer (Figure 1). This process of forming nucleosomes occurs immediately after DNA replication and is called replication-coupled nucleosome assembly.[1]

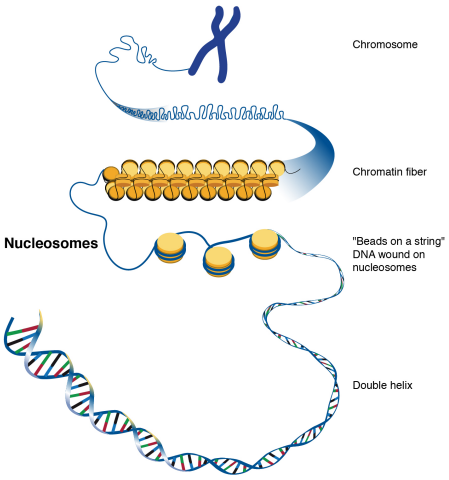

Figure 1. DNA is wrapped around a histone octamer (yellow circle) to form a nucleosome. Many nucleosomes will fit together to form a chromatin.
NHGRI, www.genome.gov

During replication-coupled nucleosome assembly, a protein known as proliferating cell nuclear antigen (PCNA) encircles the newly synthesized DNA and recruits the necessary proteins to form a nucleosome.[3] One of these proteins is chromatin assembly factor 1 (CAF-1). Once CAF-1 is bound to PCNA at the replication fork, it deposits

histones onto the DNA. The interaction between CAF-1 and PCNA is important as it is required for replication-coupled nucleosome assembly.[6] CAF-1 is thought to bind PCNA through its PCNA-Interacting peptide (PIP) motif; however, the exact mechanism through which PCNA and CAF-1 interact are not yet known.[3]

During S-phase, PCNA is post-translationally modified by the addition of a small ubiquitin-like modifier (SUMO).[2] Interestingly, CAF-1 contains a SUMO-interacting motif (SIM) downstream of the PIP motif (Figure 2). In the Dieckman lab, we hypothesize that the purpose of this SIM is to bind to the SUMO on PCNA to help recruit CAF-1 to the replication fork. Preliminary work in our lab has shown CAF-1 has a higher affinity for SUMO-PCNA than unmodified PCNA (Figure 3).

Figure 2. Illustration of the location of the PIP and SIM motif on the CAF-1 protein.

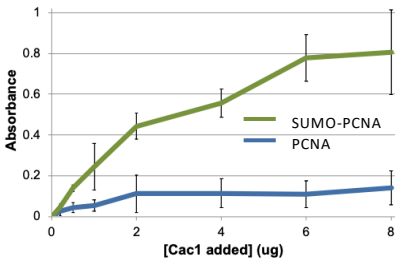

Figure 3. ELISA of CAF-1 Binding to SUMO-PCNA (green) and unmodified PCNA (blue).

My project aims to determine the structural mechanism of interaction between SUMO-PCNA and CAF-1 using small angle X-ray scattering (SAXS). In this method, purified protein (in solution) is placed in front of an X-ray beam, producing a diffraction pattern that is used to determine a myriad of structural information about the structure and dynamics. For example, we can create a P(r) plot, which describes the location of every electron in the protein complex.[4] This plot provides the D_{max} value which tells us the maximum distance two electrons are away from each other. We pair this data with molecular simulations to determine the structure of the protein

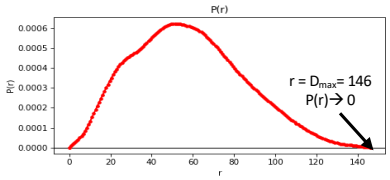

Figure 4. The P(r) plot for wildtype SUMO-PCNA-CAF-1. This shows the D_{max} is 146.

complex. We have completed SAXS for the wildtype SUMO-PCNA-CAF-1 complex (Figure 4). We thought that the PIP and the SIM could help to stabilize the SUMO on PCNA, so we developed 2 mutants: A PIP mutant and SIM mutant (Figure 5). We then completed SAXS on the mutants and found that they had larger D_{max} values than the wildtype, indicating the electrons were further apart and thus more flexible (Figure 6). Moving forward, I will be working to develop a double mutant, where both the PIP and the SIM are mutated. By running SAXS on the double mutant, we will verify that the PIP and the SIM of CAF-1 help to stabilize SUMO on PCNA. By pairing SAXS with molecular simulations, we will determine the structure of the SUMO-PCNA- CAF-1 interaction. The structure and dynamics of the SUMO-PCNA-CAF-1 complex is important in understanding how replication-coupled nucleosome assembly occurs in cells to maintain genomic integrity.

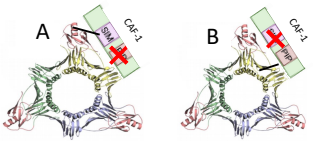

Figure 5. Illistration of SUMO-PCNA bound to CAF-1. (A) is the PIP mutant, so the PIP can't PCNA, but the SIM can bind to SUMO. (B) is the SIM mutant, where the PIP can bind PCNA, but the SIM can not bind SUMO.

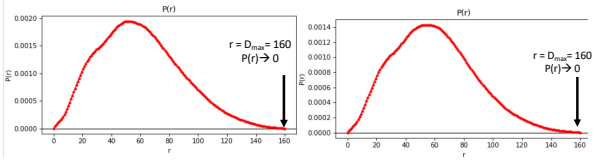

Figure 6. P(r) curve for the PIP Mutant (A) and the SIM Mutant (B). They both have higher D_{max} values than the wildtype, meaning that they have greater flexibiliy.

Citations:

1. Armstrong AA, Mohideen F, Lima CD. Recognition of SUMO-modified PCNA requires tandem receptor motifs in Srs2. Nature. 2012 Feb 29;483(7387):59-63. doi: 10.1038/nature10883. PMID: 22382979; PMCID: PMC3306252.

2. Choe KN, Moldovan GL. Forging Ahead through Darkness: PCNA, Still the Principal Conductor at the Replication Fork. Mol Cell. 2017 Feb 2;65(3):380-392. doi: 10.1016/j.molcel.2016.12.020. PMID: 28157503; PMCID: PMC5302417.

3. Kondratick CM, Litman JM, Shaffer KV, Washington MT, Dieckman LM (2018) Crystal structures of PCNA mutant proteins defective in gene silencing suggest a novel interaction site on the front face of the PCNA ring. PLoS ONE 13(3): e0193333. https://doi.org/10.1371/journal. pone.0193333

4. Powers, K. T., Gildenberg, M. S., & Washington, M. T. (2019). Modeling Conformationally Flexible Proteins With X-ray Scattering and Molecular Simulations. *Computational and structural biotechnology journal*, *17*, 570-578.

5. Smyth MS, Martin JH. x ray crystallography. Mol Pathol. 2000 Feb;53(1):8-14. doi: 10.1136/mp.53.1.8. PMID: 10884915; PMCID: PMC1186895.

6. Zhang, W., Feng, J. & Li, Q. The replisome guides nucleosome assembly during DNA replication. *Cell Biosci* 10, 37 (2020). https://doi.org/10.1186/s13578-020-00398-z

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

Dolan, M., Lovelace, J., Borgstahl, G., Dieckman, L. (2023, February 9-12). "Structure and Dynamics of an Interaction Between Proteins Involved in Gene Silencing" [poster presentation]. 7th International Nucleic Acid Conference, Cancun, Mexico.

Dolan, M., Lovelace, J., Borgstahl, G., Dieckman, L. (2022, November 12). "Novel Interaction Between Proteins Involved in Gene Silencing: Structural Studies of PCNA and CAF-1" [poster presentation]. Annual Heartland Undergraduate Biochemistry Forum at Kansas University Medical Center, Kansas City, Kansas, United States.

Dolan, M., Lovelace, J., Borgstahl, G., Dieckman, L. (2022, October 19-21). "Novel Interaction Between Proteins Involved in Gene Silencing: Structural Studies of PCNA and CAF-1" [oral presentation]. ACS Midwest Regional Meeting, Iowa City, IA, United States.

Dolan, M., Lovelace, J., Borgstahl, G., Dieckman, L. (2022, August 11). "Structure and Dynamics of a Novel Interaction Between Proteins Involved in Gene Silencing" [poster presentation]. 9th National Structural Biology and Molecular Biology Workshop, Omaha, NE, United States.

- Honorable Mention

Dolan, M., Lovelace, J., Borgstahl, G., Dieckman, L. (2022, August 7-9). "Novel Interaction Between Proteins Involved in Gene Silencing: Structural Studies of PCNA and CAF-1" [poster presentation]. NE-INBRE 20th Annual Conference Program, Nebraska City, NE, United States.

- Honorable Mention, Second Year Scholars

Dolan, M., Lovelace, J., Borgstahl, G., Dieckman, L. (2022, July 31). "Structure and Dynamics of a Novel SUMO-PCNA Interaction" [oral presentation]. 2nd Annual Genome Stability Lab Retreat, Omaha, NE, United States.

Dolan, M., Dieckman, L. (2022, April 22). "Structural Studies of the Interaction Between Proteins Involved in Gene Silencing" [oral presentation]. Nebraska Academy of Science's 132nd Annual Meeting, Lincoln, NE, United State.

Dolan, M., Dieckman, L. (2022, April 19-20). “Structural Studies of the Interaction Between Proteins Involved in Gene Silencing” [poster presentation]. Creighton University Research Week Poster Symposium, Omaha, NE, United State.

Dolan, M., Dieckman, L. (2022, April 4-8). “Using Small Angle X-Ray Scattering and X-Ray Crystallography to Investigate Structural Changes in Proteins Involved in Gene Silencing” [oral presentation]. National Conference on Undergraduate Research (NCUR), online.

Dolan, M., Dieckman, L. (2021, November 13). “Structural Studies of the Interaction Between Proteins Involved in Gene Silencing” [poster presentation]. Annual Heartland Undergraduate Biochemistry Forum at Kansas University Medical Center, online.

Dolan, M., Dieckman, L. (2021, November 13). “Mechanism of Interaction Between Gene Silencing Proteins using X-ray Crystallography” [Oral presentation]. 1st Annual Genome Stability Lab Retreat, Iowa City, Iowa, United States.

6. **Post-graduate plans (job market, graduate school, medical school, etc.)**
   Graduate School in Biochemistry

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

8. **Anticipated graduation date**
   May 2023