

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

1. Name and NetID

Olivia Nicholson (omn85932)

2. Chemistry faculty research director

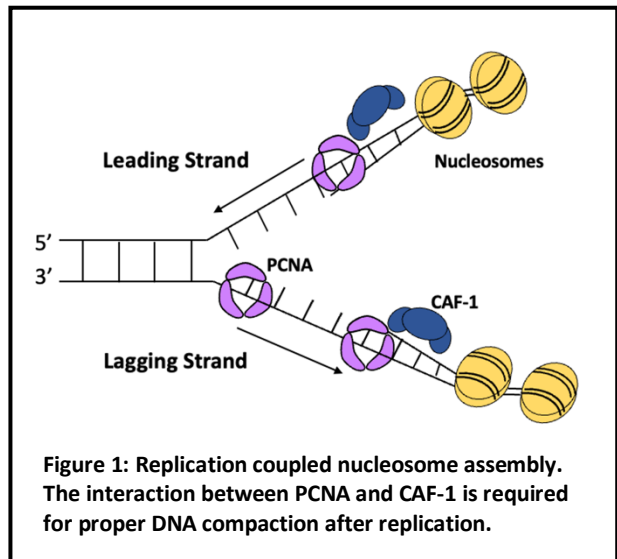
Dr. Lynne Dieckman

3. Proposal title

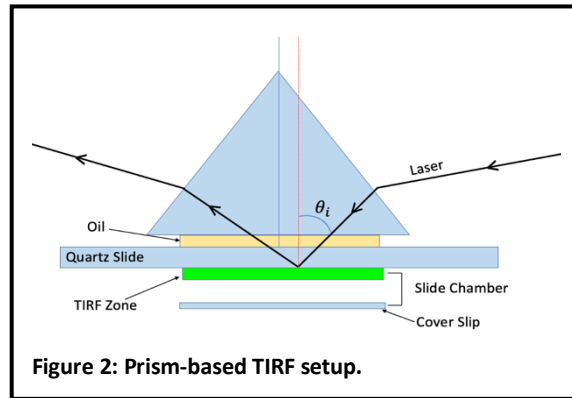
Single Molecule Studies of the Interaction between PCNA and CAF-1 Using TIRF Microscopy

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.

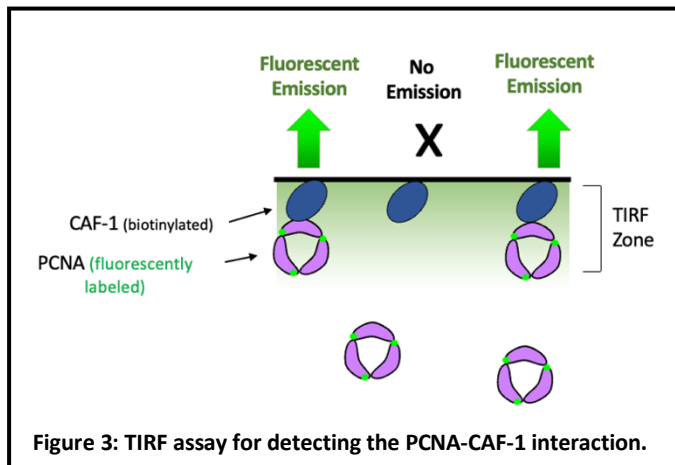
Every time a cell divides, its genome is replicated and split between two daughter cells. Immediately after replication, newly synthesized DNA is packaged in structures called nucleosomes, which are the fundamental units of chromatin. Nucleosomes are composed of double-stranded DNA wrapped around eight histone proteins. The process of packaging DNA immediately following replication, called replication coupled nucleosome assembly, is crucial for protecting DNA and maintaining genomic stability (1). Two proteins that play major roles in replication coupled nucleosome assembly are chromatin assembly factor-1 (CAF-1), a heterotrimeric histone chaperone protein that brings histone proteins to DNA for nucleosome assembly, and proliferating cell nuclear antigen (PCNA), which binds and recruits CAF-1 to the replication fork (Figure 1) (2). The interaction between PCNA and CAF-1 is essential for proper DNA synthesis and gene silencing (3). However, the molecular mechanism of binding is largely unknown. The goal of my project is to determine the kinetics of the PCNA-CAF-1 interaction. These studies will lead to a greater understanding of how this interaction promotes replication coupled nucleosome assembly to maintain proper gene silencing in cells.



I have developed total internal reflection fluorescence (TIRF) microscopy assays to measure the interaction kinetics between PCNA and CAF-1 on the single-molecule scale. Single molecule TIRF microscopy is a powerful imaging technique used to view individual fluorescently tagged molecules via laser excitation. In our prism based TIRF microscope setup (Figure 2), a laser is



a laser is shot through a quartz prism, which bends the beam of light to hit the surface of the quartz slide at the critical angle (θ). When the laser beam hits the slide at the critical angle, it is completely reflected away from the surface of the slide. The reflected light creates an electromagnetic field that extends approximately 100 nm beneath the surface of the slide, forming the TIRF zone (4). Any fluorescently tagged molecule that enters this zone will be excited, producing a fluorescent signal detectable by the microscope camera.



In my experimental setup, CAF-1 is attached to the surface of a quartz slide and fluorescently labeled PCNA is added to the slide chamber (Figure 3). The fluorescent tag on PCNA will be excited when PCNA associates with CAF-1 because CAF-1 is fixed within the TIRF zone. When PCNA dissociates from CAF-1, the fluorescence should disappear as the labeled PCNA exits the TIRF zone. The fluorescent signals are

captured via the TIRF microscope's camera and will be analyzed to determine the kinetics of the PCNA-CAF-1 interaction. Thus far, we have built a TIRF microscope system and obtained several sets of fluorescent data, suggesting we are able to visualize fluorescently labeled PCNA binding to immobilized CAF-1. Going forward with the project, I will conduct experiments with varying concentrations of PCNA and analyze the resulting fluorescence trajectories using our computer programs to determine the binding affinity of the PCNA-CAF-1 interaction.

References

1. G. Moldovan, B. Pfander, S. Jentsch, *Cell*, in press, doi:10.1016/j.cell.2007.05.003.
2. S. Smith, B. Stillman, *Cell*, in press, doi: 10.1016/0092-8674(89)90398-x
3. K. Shibahara, B. Stillman, *Cell*, in press, doi:10.1016/s0092-8674(00)80661-3.
4. E. Kudalkar, T. Davis, C. Asbury, *Cold Spring Harbor Protocols*, in press, doi:10.1101/pdb.top077800

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

Local

1) Annual Nebraska INBRE Summer Conference, Nebraska City, NE, August 7th, 2023. **Nicholson, O.**; Dieckman, L. “Single Molecule Studies of Nucleosome Assembly Proteins Using TIRF Microscopy” (poster)

2) Nebraska Academy of Sciences Annual Spring Meeting, Lincoln, NE, April 21st, 2023. **Nicholson, O.**; Dieckman, L. “Single Molecule Studies of Nucleosome Assembly Proteins Using TIRF Microscopy” (oral presentation)

3) Creighton University Undergraduate Research Fair, Creighton University, Omaha, NE, March 28th, 2023. **Nicholson, O.**; Dieckman, L. “Single Molecule Studies of the Interaction between PCNA and CAF-1 Using TIRF Microscopy” (poster)

4) Annual Nebraska INBRE Summer Conference, Nebraska City, NE, August 8th, 2022. **Nicholson, O.**; Vetro, J. “Troubleshooting an in vitro Assay for Determining Immunostimulatory Activity in Human Neutrophils” (oral presentation) - **FIRST PLACE AWARD - ORAL PRESENTATION**

5) Creighton University Undergraduate Research Fair, Creighton University, Omaha, NE. April 19th, 2022. **Nicholson, O.**; Hebert, E.; Dieckman, L. “Single Molecule Studies of the Interaction between PCNA and CAF-1 Using TIRF Microscopy” (poster)

Regional

6) Central IDeA States Regional Meeting, Kansas City, MO, June 8th, 2023. **Nicholson, O.**; Dieckman, L. “Single Molecule Studies of PCNA and CAF-1 Using TIRF Microscopy” (oral presentation) - **INVITED PRESENTATION**

7) Central IDeA States Regional Meeting, Kansas City, MO, June 7th, 2023. **Nicholson, O.**; Dieckman, L. “Single Molecule Studies of PCNA and CAF-1 Using TIRF Microscopy” (poster)

8) Heartland Undergraduate Biochemistry Forum, Kansas University Medical Center, Kansas City, MO, November 12th, 2022. **Nicholson, O.**; Dieckman, L. “Single Molecule Studies of Nucleosome Assembly Proteins Using TIRF Microscopy” (poster)

9) 6th Annual Midwest Single Molecule Workshop, University of Nebraska Medical Center, Omaha, NE, August 1st, 2022. **Nicholson, O.**; Hebert, E.; Dieckman, L. “Single Molecule Studies of the Interaction between PCNA and CAF-1 Using TIRF Microscopy” (poster)

10) Annual Midwest Genome Stability Retreat, University of Iowa, Iowa City, IA, August 15th, 2021. **Nicholson, O.**; Dieckman, L. “Single Molecule Studies of PCNA and CAF-1 Using TIRF Microscopy” (oral presentation)

National

11) National Conference on Undergraduate Research, virtual, April 5th, 2022.

Nicholson, O.; Dieckman, L. “Single Molecule Studies of Nucleosome Assembly Proteins Using TIRF Microscopy” (oral presentation)

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

After graduation, I plan to attend graduate school and work towards a PhD in pharmacology.

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

13 semesters

8. Anticipated graduation date – May 2024