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

1. Name and NetID

   Robyn Scott, RLS63839

2. Chemistry Faculty Research Director

   Dr. Lynne Dieckman

3. Title: Mapping the Binding Sites for Chromatin Assembly Factor 1 on Proliferating Cell Nuclear Antigen

4. The proposal should be limited to about 500 words and may include a few 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 these results and describe what you expect to accomplish during the time frame of this scholarship.

   Gene silencing is controlled by the specific and precise packaging of DNA in the nucleus. This process is carried out by two proteins: proliferating cell nuclear antigen (PCNA), the sliding clamp that recruits proteins to the site of DNA replication, and chromatin assembly factor 1 (CAF-1), the protein that induces DNA packaging upon its interaction with PCNA.[1] Although the interaction between PCNA and CAF-1 is known to be critical to the proper packaging of DNA, their interaction is not currently well understood. Our goal is to examine the mechanism of interaction between PCNA and CAF-1 and how this interaction induces gene silencing.

   PCNA interacts with over 50 different proteins during DNA replication, repair, and recombination, and previous studies have identified the specific region of PCNA to which almost all of these PCNA-interacting proteins bind.[2] However, our preliminary data suggest that CAF-1 may interact with PCNA at a novel, secondary site in addition to the canonical protein-binding site.[3] If this is the case, it could help to explain how PCNA discriminates between its many binding partners and recruits CAF-1 to DNA and induces gene silencing. To determine if this is in fact a secondary site of interaction for CAF-1, another student and I have performed site-directed mutagenesis of five amino acids that we hypothesize are critically important to this novel CAF-1 binding site on PCNA. We purified the mutant PCNA proteins and have carried out protein binding studies (enzyme-linked immunosorbent assays (ELISAs)) with purified CAF-1 and both wild type and mutant PCNA proteins. These preliminary binding assays suggest that the mutant PCNA proteins show varied affinities for CAF-1, suggesting that the mutated amino acids are likely involved in the proposed secondary site of interaction between PCNA and CAF-1 (Figure 1). Of particular interest, one of the mutant PCNA proteins (S43A) showed increased binding affinity to CAF-1 in

comparison to wild type PCNA, as seen in Figure 1A. This is interesting because in cells, this mutation could cause a global increase in gene silencing.

In order to better quantify the functional consequences of each mutation in PCNA, I have recently begun to develop a fluorescence anisotropy binding assay. Here, polarized light is used to excite fluorescently tagged CAF-1. When unbound, CAF-1 has a low anisotropy as it is free to rapidly rotate and reorient itself before emitting light. As increasing amounts of PCNA are added, the anisotropy increases because CAF-1 is no longer able to rotate and reorient itself as rapidly. This assay can be used to determine the binding constant ($K_d$) for each of the PCNA proteins and CAF-1. During the time frame of this project, I will continue to develop and optimize the fluorescence anisotropy assay in order to determine the $K_d$ values for CAF-1 and both the wild type PCNA and mutant PCNA proteins. This will enable us to determine which amino acids in the proposed secondary site of interaction between PCNA and CAF-1 are critical for their interaction and will help us to understand their mechanism of binding. Understanding the interaction between PCNA and CAF-1 will improve knowledge of and potentially lead to manipulation of gene silencing.

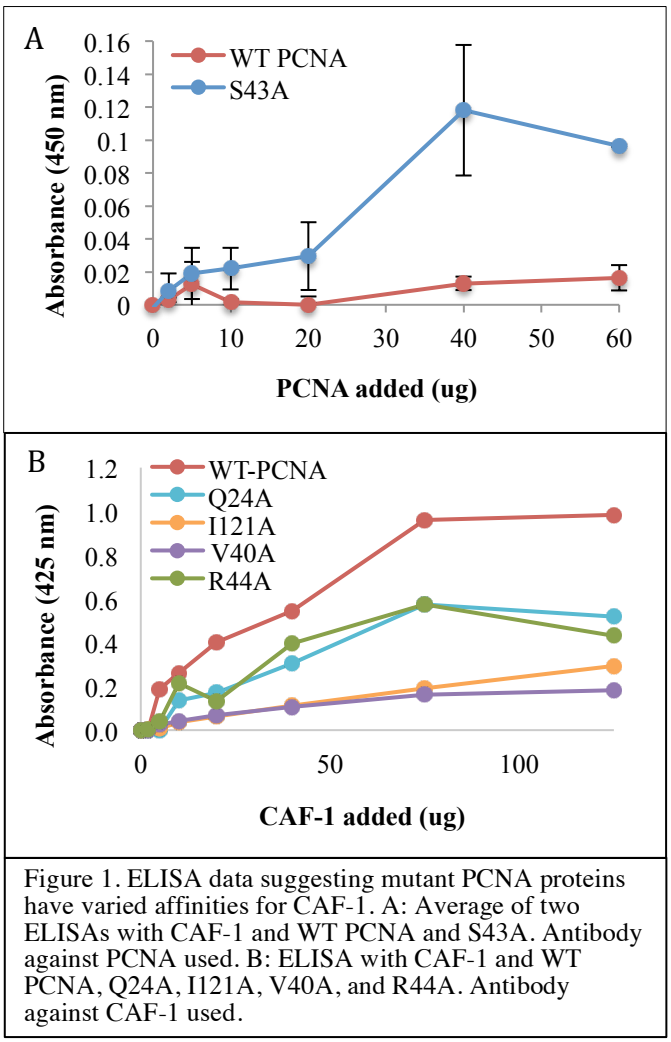


Figure 1. ELISA data suggesting mutant PCNA proteins have varied affinities for CAF-1. A: Average of two ELISAs with CAF-1 and WT PCNA and S43A. Antibody against PCNA used. B: ELISA with CAF-1 and WT PCNA, Q24A, I121A, V40A, and R44A. Antibody against CAF-1 used.

References

1. Z. Zhang, K. Shibahara, B. Stillman, PCNA connects DNA replication to epigenetic inheritance in yeast. *Nature*. 408, 221-225 (2000).
2. G. Maga, U. Hübscher, Proliferating cell nuclear antigen (PCNA): a dancer with many partners. *Journal of Cell Science*. 116:15, 3051-3060 (2003).
3. C. Kondratick, J. Litman, K. Shaffer, M. Washington, L. Dieckman, 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 (2018).

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

   Future presentations:
   15th Annual Honors Day, April 2019
   Nebraska Academy of Sciences Annual Meeting, April 2019

   Past presentations:
   Scott R and Dieckman L. Mapping the binding sites for chromatin assembly factor 1 on proliferating cell nuclear antigen. Poster session presented at: NE-INBRE 16th Annual Conference; 2018 August 5-7; Nebraska City, NE.

   Scott R and Dieckman L. Mapping the binding sites for chromatin assembly factor 1 on proliferating cell nuclear antigen. Oral presentation at: Nebraska Academy of Sciences Annual Meeting; 2018 April 20; Lincoln, NE.

   Scott R, Shaffer K, Ho J, VanDolah H, Carrig M, and Dieckman L. Identification of PCNA binding sites on chromatin assembly factor 1. Poster session presented at: Biophysical Society 62nd Annual Meeting; 2018 February 17-21; San Francisco, CA.

   Scott R and Swanson P. Evidence for IL-10 mediated suppression of BAFF. Oral presentation at: NE-INBRE 15th Annual Conference; 2017 August 6-8; Nebraska City, NE.

   Scott R, Barrera C, Graft W, Shikiya R, and Soto P. Dynamics of residue contacts in prion proteins influence transmission barrier across species. Poster session presented at: NE-INBRE 14th Annual Conference; 2016 August 7; Nebraska City, NE.

   Publications:
   Palmer, V., Worth, A., Scott, R., Perry, G., Yan, M., Li, Q., and Swanson, P. (2018). IL10 restrains autoreactive B cells in transgenic mice expressing inactive RAG1. *Cellular Immunology*. 331, 110-120.

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

   I am planning on working as a research assistant for one year before matriculating to an MD/PhD program. I plan to apply for the NIH intramural post-baccalaureate program, but am also pursuing a potential opportunity to continue doing research at Creighton during my gap year. For the MD/PhD program, I am interested in studying immunotherapy for cancer treatment, potentially from a biochemistry approach focusing on protein interactions.

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

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8. Anticipated graduation date:

   May 2019

Applicant signature

Chemistry research director's signature