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

1. Name and NetID

   Alexa Schmitz aes36747

2. Chemistry Faculty Research Director

   Dr. Lynne Dieckman

3. Title: Maximizing Stability of PCNA in long term storage conditions
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.

Proliferating Cell Nuclear Antigen (PCNA) is a homotrimeric ring-shaped protein that is important for DNA replication by acting as a sliding clamp and recruiting polymerases so that they can bind to it and synthesize DNA.[1] Some other processes that PCNA is involved in are: DNA replication, chromatin assembly and remodeling, base and nucleotide excision repair, and sister-chromatid recombination.[1] Since PCNA is important in many processes, it is a widely studied protein that researchers work with daily. Purifying PCNA is relatively straightforward; however, purified PCNA is extremely unstable. It is only active for about a week when stored at a temperature of 4°C. In order to not purify PCNA every week, researchers often store PCNA at -80°C or -20°C. When PCNA is retrieved from the -80˚C freezer, column chromatography is used to "revive" the PCNA to increase the stability and activity which is very expensive and time-consuming process. If we were able to identify better storage conditions for PCNA, these techniques would not have to be done, thus increasing productivity for research projects and saving time for researchers. The goal of my project is to identify storage conditions that maximize the stability of PCNA when in long-term storage. To accomplish this goal, I will be purifying PCNA, freezing the protein at -80˚C in different conditions (Table 1), and testing

Table 1: Eight Conditions for ELISAs

Condition Number	Buffer (50mM, pH=7.4)	Percent glycerol	Additive or Special Condition
1	$NaPO_4$	10%	5 mM DTT
2	$NaPO_4$	10%	5 mM DTT, 0.2 mM EDTA, Protease inhibitors
3	$NaPO_4$	25%	5 mM DTT
4	$NaPO_4$	25%	5 mM DTT, 0.2mM EDTA
5	$NaPO_4$	25%	5 mM DTT, 0.2 mM EDTA, Protease Inhibitors
6	$NaPO_4$	25%	5 mM DTT, Protease Inhibitors
7	Tris	25%	5 mM DTT, 0.2 mM EDTA
8	Tris	25%	5mM DTT

the activity of PCNA over a period of time using enzyme-linked immunosorbent assay (ELISAs).

We have identified some components that we predict could increase the stability of stored PCNA. These include adding: 1) higher concentrations of glycerol, 2) higher concentrations of dithiothreitol (DTT), 3) adding EDTA, 4) and/or protease inhibitors. Glycerol is a cryo-protectant which helps prevent damage to the proteins by preventing ice crystals forming in solution.[2] Metal ions bind to metalloproteases which break down peptide bonds in proteins.[3] EDTA binds to these metal ions, which then inhibits them from binding to proteases and therefore could increase protein stability.[2] DTT is a reducing agent that reduces the formation of disulfide bonds of molecules.[4] Non-native disulfide bonds affect the folding of proteins, which would affect their stability.[5,6] Protease inhibitors inhibit all types of proteases, which break peptide bonds of proteins if present in solution.[7]

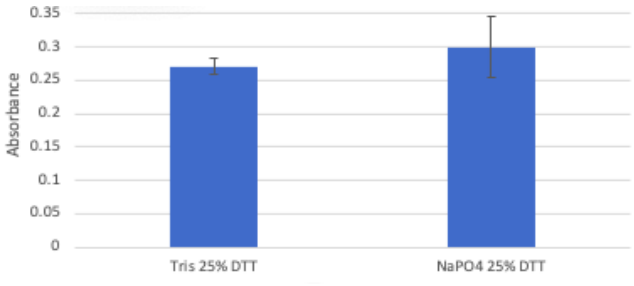

Figure 1: ELISAs using conditions 3 and 6. In ELISAs, PCNA in their respective conditions are added to wells, then a secondary protein is added which PCNA binds to. Next, GST antibody is added to each well since the secondary protein is GST tagged. Lastly, O-phenylenediamine is added to the wells, which changes color in the presence of GST antibody. The absorbances of these wells are read on a plate reader and graphed to compare conditions.

Since the start of this project in the fall of 2019, I have determined that PCNA has better binding activity when stored in sodium phosphate buffer ($NaPO_4$) than in tris buffer (Figure 1). This could be because the pH of Tris buffer decreases as temperature increases.[8] This change in pH could affect the stability of PCNA in the freezer.

I will be performing an ELISA assay with the PCNA stored in the different conditions. In addition to the ELISA assays, I will be performing a native polyacrylamide gel electrophoresis (PAGE), which separates proteins based on size, shape, and charge. [9]A native PAGE will be conducted with the eight conditions to determine if PCNA is aggregated or non-trimeric. From these studies, we will learn which storage conditions are the best for the binding activity and stability of PCNA.

1. Moldvan, G. L.; Pfander, B.; Jentsch, S. PCNA, the Maestro of the Replication Fork. *Cell.* **2007,** *129*, 665-669.
2. T. S. Inc. http://tools.thermofisher.com/content/sfs/brochures/TR0043-Protein-storage.pdf (accessed November 2, 2020).
3. Tallant, C; Marrero, A.; Gomis-Rüth, F. X. Matrix metalloproteinases: Fold and function of their catalytic domains. *Biochimica et Biophysica Acta-Molecular Cell Research.* **2010,** *1803*, 20-28.
4. Lukesh III, J. C.; Palte, M. J.;. Raines, R. T. A Potent, Versatile Disulfide-Reducing Agent from Aspartic Acid. **2012,** *134*, 4057-4059.
5. Kosuri, P.; Alegre-Cebollada, J.; Feng, J.; Kaplan, A.; Inglés-Prieto, A.; Badilla, C. L.; Stockwell, B.R.; Sánchez-Ruiz, J.M.; Holmgren, A.; Fernández, J. M. Protein folding drives disulfide formation. *Cell.* **2012**, *151*, 794-806.

6. Deller, M. C.; Kong, L.: Rupp, B. Protein stability: a crystallographer's perspective. *Acta crystallographica Section F, Struct. Bio.Comm.* **2016,** *72*, 75-95.
7. López-Otin, C.; Bond, J. S. Proteases: Multifunctional Enzymes in Life and Disease. *Jour. Bio. Chem.* **2008,** *283*, 30433-30437.
8. Reineke, K.; Mathys, A.; Knorr, D. Shift of pH-Value During Thermal Treatments in Buffer Solution and Selected Foods. *Intl. Jour. Food Prop.* **2009,** *14*, 870-881.
9. Li, C. M.; Miao, Y.; Lingeman, R. G.; Hickey, R. J.; Malkas, L. H. Partial Purification of Megadalton DNA Replication Complex by Free Flow Electrophoresis. *PLOS One.* **2016,** *11*, e0169259.

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

   Clare Boothe Luce Seminar (2021)

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

   Graduate School

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 2021

Applicant signature

Chemistry research director's signature