PEW RESEARCH PROPOSAL

1. **Title of the project**.

Protein Arginine Methyltransferase 1 R353P mutation affects dimerization in colorectal cancer.

2. **Statement of the end product(s)** (book, chapter, article, play composition, exhibit, or other dissemination means appropriate to your discipline).

   This research will be presented by Chemistry and/or Biochemistry students at the annual American Chemical Society meeting in the spring of 2027. These results will be published in a peer-reviewed scientific journal. Additionally, this research will help me mentor undergraduate students majoring in Chemistry and Biochemistry, train them in Biochemical techniques, and help them see the undeniable link between the Christian faith and the natural world.

3. **Explanation of the scholarly activity.**

   I. Statement of the scholarly activity.

   My research project focuses on proteins that catalyze the modification of other proteins through methylation, known as Protein Arginine Methyltransferases (PRMTs). In this particular project, I aim to test a PRMT1 colorectal cancer mutant for its methyltransferase activity. I hypothesize that the activity of this mutant is diminished due to incorrect protein assembly caused by the mutation.

   Proteins are large, complex molecules that play a key role in virtually all the cell's operations. Arginine methylation is an important modification of proteins, involved in many cellular processes like cell signaling, cell division, and even viral replication. In recent years, the significance of PRMTs in human diseases has been increasingly studied, especially in cancer and cardiovascular disease. Although the importance of these enzymes is recognized, the understanding of exactly how PRMTs function is still limited. My aim is to better understand the mechanisms by which these functions operate and to elucidate how they are regulated. By studying the crystal structure of PRMT1, the major isoform of PRMTs in humans, we have found two oppositely charged amino acids that seem to be interacting and might play a role in stabilizing the active conformation of the enzyme. Interestingly, one of these amino acids has been reported to be mutated in colorectal cancer. I hypothesize that this mutation is disrupting the native conformation of the protein, and therefore also the protein's activity, and I would like to test this hypothesis. This knowledge is crucial for the design of new drugs that would help us target these proteins in the diseases they are involved in.

This grant would enable me to purchase a radiation-free methyltransferase assay kit and a customized peptide to test the methyltransferase activity of the protein variants created, and compare them to the wild-type (naturally occurring) version of PRMT1.

II. Description of the activity and its goals.

- Develop a radiation-free methyltransferase assay to test the methyltransferase activity of the colorectal cancer variant.
- Run the protein samples on native gels and size exclusion chromatography to determine the oligomeric state.
- Perform site-directed mutagenesis to create additional protein variants.
- Express and purify the protein variants.

III-IV. Theoretical framework and Brief examination of scholarly literature or context of the activity within your discipline.

The modification of proteins is a tool used by cells to react to changes or events in their environment. These marks expand the structural and functional diversity of the proteome. One such post-translational modification, methylation, can occur on amino acids such as lysine, arginine, histidine, or proline, and has also been found on carboxy groups (1). Arginine methylation is a common post-translational modification that functions as an epigenetic regulator of transcription and plays key roles in mRNA processing, DNA damage repair, mRNA translation, cell signaling, and cell fate decision (2-4). This modification is carried out by the family members of the protein arginine methyltransferases (PRMTs). The PRMTs transfer a methyl group from the donor molecule S-adenosyl-L-methionine (AdoMet) to the basic amino acid arginine in the substrate protein. The substrate arginine residue can be methylated in three distinct ways on the guanidino group, forming three different products, according to which the PRMTs can be classified into three types. Type I, type II, and type III enzymes all catalyze the formation of monomethyl arginine (MMA). Type I PRMTs (PRMT1, 2, 3, 4, 6, and 8) further catalyze the production of asymmetric dimethylarginine (ADMA), and type II PRMTs (PRMT5, PRMT9) further catalyze the formation of symmetric dimethylarginine (SDMA) (5). PRMT7 is only capable of forming MMA, and it is the only known type III PRMT (6-9). The main result of arginine methylation is the alteration of protein-protein or protein-nucleic acid interactions. This alteration arises primarily from changes in hydrogen bonding to the methylated arginine residue (10).

PRMT1 was the first mammalian protein arginine methyltransferase to be cloned and characterized (11). This enzyme is responsible for the majority (about 85%) of total protein arginine methylation activity in mammals (12). PRMT1 methylates histone H4 at arginine 3, depositing an ADMA mark, activating transcription, and thus contributing to the histone code (13). In prostate cancer, the dimethylation of histone H4R3 catalyzed by PRMT1 has been associated with transcriptional activation

and positively correlates with prostate tumor grade, which is associated with increased abnormality and an increased likelihood of tumor growth. As a consequence, this mark can be used to predict the risk of prostate cancer recurrence in patients (14). Additionally, when H4 arginine 3 is asymmetrically dimethylated, the methylarginine recognizing molecule Tudor domain-containing protein (TDRD3) interacts with this mark. TDRD3 has been identified as one of the candidate genes for a scoring system of breast cancer as higher expression of this gene is linked to lower survival rates of patients with this condition (15,16).

It has been shown that a mutated nonfunctional PRMT1 gene in mice leads to embryonic lethality. This loss leads to spontaneous DNA damage, cell cycle progression delay, checkpoint defects, aneuploidy, and polyploidy. These data show that PRMT1 is required for genome integrity and cell proliferation, and that arginine methylation is a key posttranslational modification in the DNA damage response pathway in proliferating mammalian cells (17). Endothelium-derived nitric oxide (NO) is a potent vasodilator that plays a critical role in maintaining vascular homeostasis. Altered NO biosynthesis has been implicated in cardiovascular disease (18). Evidence from animal models and clinical studies suggests that asymmetric dimethylarginine (ADMA) and monomethyl arginine (MMA) are nitric oxide synthase inhibitors. These products contribute to reducing NO generation and to disease pathogenesis (19). In pathological conditions such as pulmonary hypertension, coronary artery disease, diabetes, and hypertension, plasma ADMA levels have been shown to increase above average concentrations (20-23). Moreover, it has been shown that ADMA levels are a good prediction of cardiovascular mortality in patients who have coronary heart disease (24). PRMT1 plays a critical role in these cases because it is the primary enzyme in the formation of ADMA-containing proteins. Therefore, understanding all of the intrinsic and extrinsic factors that contribute to the regulation of PRMT1 activity and the production of ADMA by PRMT1 is crucial.

The significant role that protein arginine methyltransferases play in biological pathways and diseases has made the PRMTs prominent targets for the development of inhibitors that could serve as therapeutic agents. This surge towards inhibitor design (10,25) has reinforced the need for a complete understanding of the specific mechanisms by which the different PRMTs operate. Importantly, proteins harboring each of the modified arginines can be biologically distinct (26,27). Another layer of complexity exists when a substrate has more than one target arginine, which could lead to a combinatorial set of modified proteins. Thus, characterizing the kinetic mechanism of the PRMTs and deciphering how the final methylation state of the substrate arginine is determined are crucial in order to fully understand how this family of proteins functions and can be controlled or inhibited.

In a study intending to provide knowledge about amino acid residues that are key for PRMT1 activity, the Hevel lab at Utah State University previously reported the automethylation characteristics of the M48L PRMT1 mutant (*15*). After this study, it was hypothesized that Arg-353 (R353) was the site of automethylation. To confirm these findings, the M48L-R353K PRMT1 variant was generated. Automethylation assays were performed, and R353 was confirmed as the site of automethylation. Additionally, the methyltransferase activity of the enzyme was greatly reduced.

Because the overall methylation rates were decreased in the double variant M48LR353K when compared to the single M48L variant, it was hypothesized that residue R353 was also important for

PRMT1 catalysis. To test this hypothesis, a single mutant R353K-PRMT1 was also generated. This mutation severely affected the activity of PRMT1, as seen by the significantly decreased methylation rate.

Later, Dr. Orlando Acevedo, a computational chemist at the University of Miami, modeled the N-terminus helix, which is not seen in the PRMT1 structure (PDB 1OR8), using the PRMT3 (another type I PRMT) structure as a guide. Using this modeled structure, the observation was made that the R353 of one PRMT1 monomer seems to be forming an ionic bond (salt bridge) with D37 of another monomer. Therefore, we hypothesized that the disruption of this bond might be affecting PRMT1 dimerization, which is necessary for activity, causing the low activity that we see in the R353K single mutant(29, 30). Interestingly, after these observations, we have also found that the R353 residue has been reported to be mutated in colorectal cancer to a proline. Recent studies by the Hevel lab have shown that many cancer-associated mutations of PRMT1 disrupt the oligomerization of this enzyme (30). We believe that this mutation has the same effect on the protein, disrupting its native conformation as well as its overall activity. To test this, the R353P mutant was created, expressed, and purified in my lab. Size-exclusion chromatography and native gel assays can be used to test if the dimerization of the enzyme is affected by changing these charges. Then, a methyltransferase assay can be used to determine if this also correlates with the decrease in methyltransferase activity. It is important to highlight that R353 may be one of the first residues identified to control activity outside of the active site, pointing to a novel mechanism of controlling PRMT1 catalysis. A study using molecular dynamics to study the communication pathways and allostery between PRMT1 monomers suggested that R353 might have an effect in long-range communication. Additionally, this study suggested that this residue may play an important role in the binding of positively charged substrates, which also underscores the potential value of studying this amino acid residue (31).

Understanding the key residues that regulate the activity of these proteins can help in the development of new drugs to target those diseases. The funds obtained through this grant would also help me mentor undergraduate research students at Union University who plan to enter health professions and scientific careers. This research will be later presented at the national American Chemical Society conference by students in the Chemistry department and published in a peer-reviewed scientific journal.

References:

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V. Connection to Ernest Boyer's Model of Scholarship.

This project relates to Ernest Boyer's categories of the scholarship of discovery, integration, application and teaching.

Scholarship of discovery: The project will advance our understanding of PRMTs, the way they function, and how they are related to disease, especially colorectal cancer.

Scholarship of integration: This project integrates knowledge from biochemistry, structural biology, and molecular biology.

Scholarship of application: The findings of this project will contribute to the understanding of the key residues that regulate the activity of these enzymes, which in turn can be used in the development of new drugs to target the diseases these enzymes are involved in.

Scholarship of teaching: The results of this study will be presented and shared in various academic settings, including a peer-reviewed journal and oral presentations at American Chemical Society national meetings.

4. **An essay (500 - 1000 words) describing how the Christian faith relates to your understanding of your discipline and how it relates to this scholarly endeavor.**

Biochemistry is a scientific discipline that describes the structures, mechanisms, and chemical processes shared by all living organisms and how their remarkable properties arise from thousands of different biomolecules.

Thinking Christianly about biochemistry should come naturally to anyone. This is because the first thing we learn about the Judeo-Christian God in the book of Genesis is that he is the Creator of all things. Later, in the New Testament, in the book of Colossians, Chapter 1, verses 16-17, we learn that not only all things exist through the creative power of the Lord, but in him all things continue to be held together in existence. Also, in the book of Romans Chapter 1 and verse 20, the apostle Paul tells us that his invisible attributes, namely, his eternal power and divine nature, have been clearly perceived, ever since the creation of the world, in the things that have been made. Therefore, the bible teaches us that we can know certain things about God naturally by the things that have been made.

The belief that Christianity is at war with science is a myth. I am in the same camp as many founders of early modern science, like Newton, Boyle, and Kepler, who believed that the testimony of nature actually supports the Judeo-Christian worldview. This means that scientific evidence and Christian belief support each other when they are correctly interpreted. We can think about the Bible and science

as mutually enforcing revelations of the same God. One revelation, infallible (the Bible, God's word), and the other limited by human knowledge and human interpretation.

Biochemistry is one of many scientific fields where the beauty and complexity of living systems are clearly shown. One of the things my students learn in my classes is how biomolecules and biological pathways are designed. In class, we also see how there is an outstanding chemical logic that makes these complex, sophisticated, and very well-orchestrated processes even stronger. One thing that my students should recognize, and I emphasize from day one of our class, is that amid all of that complexity and chemical diversity in nature, there is a set of principles that dictates the architecture and operation of these molecules and these pathways. It is one of my tasks in my classes to help my students see that intricate biological systems conform to a simple set of rules, and that only a good designer who exercises thought and care could orchestrate these very effective systems. After understanding this, I believe it becomes reasonable to believe that an intelligent Creator is responsible for the design, origin, and history of life as described in Genesis.

When working on biochemical research with the students I mentor, we perform laboratory procedures designed to measure the activities of biomolecules and biochemical systems. To get our experiments to work properly, we spend most of our time in the lab carefully designing and optimizing each test before executing it with exacting precision in the laboratory. Optimizing these assays is not easy. In fact, it could take weeks or even months of painstaking effort to get the experiments to work just right.

As I teach my research students in my lab how to design and optimize biochemical procedures, I would like for them to experience the fact that optimized systems don't just happen by chance, whether they are laboratory experiments, or well-designed cars or computers. Instead, optimization will always result from the mind and efforts of intelligent agents like themselves and therefore serves as a sure indicator that we were created in the image of a great designer.

In this specific project, we are studying an enzyme that was carefully designed and examining what happens when a single (among 353) amino acid is mutated in cancer. I would like my research students to see how each of those amino acids was put together in their specific place with a purpose. And how modifying just one or two amino acids can completely alter the function that the protein was designed to have.

I believe that both in class and in the lab, Biochemistry provides a unique opportunity to observe the splendor and elegance of life's Chemistry, which should convince anyone that a good and perfect Creator is behind the origin, purpose and meaning of life.

5. **A time frame for the completion and a plan for the dissemination of the project.**

Summer 2026

- Purified R353P variant assayed through native gels
- Methyltransferase activity of the variant is assayed using MTase Glo kit
- More protein variants are created using site-directed mutagenesis

Fall 2026
- Manuscript is prepared

Spring 2027
- Manuscript is submitted
- Results presented at the national meeting of the ACS

6. **A brief budget**

MTase glo assay	600.00
96 solid white well plates	200.00
H4-21 TFA free custom peptide	550.00
Refurbished Microplate reader	3150.00

7. **A current *curriculum vitae*.**

CV has been attached

8. **Two letters of recommendation should be submitted directly to chair of the Research Committee attesting to the worth of the project, the candidate's scholarly competence, and his/her ability to complete the project with distinction in a timely fashion.**

Dr. Joan Hevel and Dr. William Thierfelder

9. **Two letters of support (from Department Chair and Dean) should be submitted directly to the chair of the Research Committee stating their approval of the proposed project, sharing any potential departmental/college/school support the candidate may receive if the Pew Grant is awarded for their proposal (e.g., course release time or allocation of professional development funds) and confirming their confidence in the candidate's ability to complete the proposed project in a timely manner.**

Dr. Fred Johnson and Dr. Mike Salazar