RESEARCH PROPOSAL HOW-TO

There are three essential components of writing a research proposal in less than 5 pages (for longer research proposals, more sections may be required).

Introduction

- Conduct a literature review of your topic that addresses the following:
    - 1. What is your research about? Give us some context. Summarize in 3-5 sentences
        - e.g. Wnts are short-range signaling proteins that are important during cell differentiation, renewal, and propagation. Wnts are expressed by ribosomes in the endoplasmic reticulum. After translation, the proteins are palmitoleated in the ER by membrane-bound O-acyltransferase (MBOAT) porcupine (PORCN). This post-translational modification makes Wnt hydrophobic and therefore reliant on other proteins and endosomes to be carried within the hydrophilic cellular environment. Wnt binds to the dedicated transmembrane carrier protein Wntless (WLS) that carries Wnt from the ER to the plasma membrane through the Golgi apparatus. Then, Wnt is released and binds to receptors on neighboring cells like Frizzled which has a cysteine-rich domain (CRD) that is crucial for Wnt binding and may serve an additional purpose of monitoring the amount of extracellular Wnts.
    - 2. Why do we care about this topic? Summarize in 2-3 sentences.
        - e.g. **Dysregulation of the Wnt signaling pathway can cause developmental issues and oncogenesis, particularly in colorectal cancer. A missense mutation in WLS, the dedicated carrier protein for Wnt, can cause Zaki syndrome, a developmental disorder marked by skeletal and multi-organ defects.**
    - 3. What is the experimental method used to study this topic? Summarize in 1 sentence.
        - e.g. This study proposes the structural elucidation of apo WLS to understand how one dedicated carrier can accommodate 19 different proteins and provide insight into how anti-cancer therapeutics can fit into the binding pocket.

Writing Aims

- Propose a list of 3-5 aims (usually 3 is a good number for a short proposal, but subdivide if needed)
    - Step 1: Title your aim with your intended group of experiments (should be broad enough that you can elaborate in detail in the subsection paragraph)
        - e.g. Aim 1. Preliminary Screening of WLS to optimize yield in small-scale
    - Step 2: Aim to be as specific as possible with your procedures.
        - e.g. In order to maximize the yield of WLS to visualize the protein on a cryo-EM grid, I will optimize protein production, purification, and stability. These parameters will be established first in small-scale. First, a viral titration experiment should be performed to optimize the production of WLS in HEK293S GNTI- cells, a type of cell lacking N-acetylglucosaminyltransferase I that enhances membrane protein expression. The WLS virus was produced from pEG BacMam vector and amplified in increasing volumes of Sf9 cells. In the viral titration experiment, a small-scale purification of WLS on nickel resin (~200 mL

cells per condition) will be conducted with cells infected at four ratios (e.g., 1:100, 1:80, 1:50, and 1:20). The eluate will be run on an SDS-PAGE gel to determine the optimal viral ratio while also conserving virus. If needed, the baculovirus will be amplified in a greater volume of Sf9 cells. In tandem, synthetic Fabs binding to WLS will be used to help visualize WLS in the apo state as WLS is a relatively small protein with few features. I will conduct Fab binding experiments; these will be done using a small-scale purification of WLS on nickel resin and the eluate will be reconstituted in nanodisc to be run on a high-performance liquid chromatography column (HPLC) to determine if there is Fab binding to the protein. For the purposes of purifying WLS on a large scale to freeze on cryo-EM grids, a nanodisc screening will be conducted; different ratios of membrane-scaffold protein (MSP, for example 1E3D1) and lipid (e.g., POPG) will be used. Once the nanodisc reconstitution has been optimized, the reconstituted sample will be further purified by HPLC and samples will be run on an SDS-PAGE gel to check for purity.

Future Studies

- Conclude your proposal with how this experimental information will be used going forward + what future work can be done in this field
    - e.g. To verify the apo WLS structure, I'd use the validation tests within cryoSPARC to confirm the quality of the micrographs and check for overfitting. Additionally, I would compare the apo structure to the bound ligand structure to see if major helices are conserved (we predict a slight shift in the conformation between the bound and unbound structures). I'd also try to cross-link two of the transmembrane helices to see if the binding pocket, upon cross-linking, is inaccessible to the ligand (thus attempting to support our hypothesis of an "open," unbound conformation for WLS). Of course, we can never fully know if a protein adopts its conformation solved via cryo-EM in actual human physiology, but these steps can be taken to have more confidence in the biological relevance of the structure.