I am a high school junior currently working on an Arabidopsis Thaliana project, where I'm specifically looking at the Jasmonate-mediated immune response system. The objective of the project is to keep the JA system "open" under any and all types of pathogen stress. Let me explain what that means: certain pathogens such as P. syringae or P. viticola "mess with" the JA system and prevent JAZ proteins from being degraded. JAZ proteins under "normal" pathogen stress (pathogens that don't mess w/ the JA system) are ubiquitinated for degradation through 3 key players, jasmonoyl-L-isoleucine, a skp1-cullin-box e3 ubiquitin ligase with a COI1 receptor complex, and a generic 26S proteasome. JA-ile biosynthesis and the SCF ubiquitin complex's activity are known to be inhibited with certain pathogens, making it impossible for jaz proteins to degrade, meaning MYC transcription factors remain inhibited and unable to recruit MED25 and the pre-initiation complex that recruits rna polymerase 2 never completely forms → transcription never begins. The goal of this project is to allow transcription of those defense genes to begin and continue under all types of pathogen stress. I considered trying to restore the 3 key players' (mentioned above) "normal activity" but determined that would be far too difficult. Instead, I came to this unique idea: if I could use a pathogen inducible Crispr/cas13 system to modify the mRNA of MYC transcription factors to target key amino residues in the jasmonate-interacting-domains of the MYCs to PREVENT JAZ proteins from binding to MYCs, I could in theory keep the JA system "open". Now there were concerns regarding how this MYC mutagenesis could affect cross-talk with other immune systems. I'm stating now that cross-talk won't be an issue. NPR1 is another key inhibitor that inhibits MYC activity - it is activated by the SA system and similar to JAZ proteins, physically binds to MYC to prevent MED25 recruitment (and thereby prevents rna pol 2 recruitment). The key thing to remember is that I am JUST not trying to keep the JA system open, I am trying to RESTORE NATURAL natural and normal immune responses, SO if the NATURAL response WANTS MYC to be inhibited, then let that be so! Through mutagenesis of the MYC, are merely ensuring that WHEN (of if) the plant decides that it wants MYC to be activated (SA system stops NPR1 production), there is not an added layer of inhibition on MYC (jaz proteins are binded → need to be lysed → can’t be lysed b/c inhibition → I already prevented them from binding so no inhibition on MYC after NPR1 is lifted → if SA system wants to transfer immune response to JA system then there won’t be any issues). In terms of other crosstalk, these small molecules become relevant: WRKY, miR160, GSK3. However these small molecules, once again, won’t matter. Here’s why: if a plant, eg.

arabidopsis thaliana, is under attack, Pattern triggered immunity and effector triggered immunity will eventually result in either induced systemic response or systemic acquired response, meaning the entire plant is aware of the fact that it is under attack, thus pre-priming promoters of defense genes (meaning promoters of growth genes are going to be hidden from MYC and other transcription factors), so in theory "ja-inhibiting" crosstalk will ALREADY be limited. In sum, the crispr/cas13 mutation on MYC TFs is focused on a specific regulatory point (in the overall immune response system) and shouldn't raise immediate concerns regarding direct interference with the other molecular bio-entities (molecular bio-entities = AP2/ERF/MYB, NAC, TGA transcription factors, glucosinolates/phytoalexins/lignins/flavanols (just all those secondary metabolites), and kinase activity). So cross-talk is not a concern, but about the actual effect? → Would a JID mutation ACTUALLY allow MYC to recruit RNA poly 2 if the plant has now decided it wants the downstream JA defense genes activated? What I'm talking about here is factors such as chromatin remodeling. NINJA and TPL/TPR co-repressors work in physical combination with JAZ proteins to inhibit MYC (it should be noted that only JAZ is directly binded to MYC). HDACs (histone de-acetylase enzymes) are recruited by NINJA/TPL and help keep the coding gene (the actually extein/intein coding regions of DNA downstream of the promoters that MYC is binded to) inactive (by simply keeping acetyl groups out). BUT, HDACs should in theory not be fully recruited if JAZ never physically binds to MYC. And assuming our CRISPR/Cas13 is designed to minimize off-targeting, there really should not be any concerns regarding feasibility of this project.

For now I'll focus on a specific MYC, MYC2 (I will look at MYC3/4 later as those 2 have potentially more cross-talk implications and MYC2 is a good starting point). The project is going to be divided into 2 phases, a dry lab phase and a wet lab phase, with the in silico dry lab phase being the actual experimentation and the wet lab phase being actual in vivo validation. The dry lab phase will come first and include:

1. 3D Modeling of MYC2:
    - Use existing structural data or homology modeling to create a 3D model of MYC2 in silico.
        i. Develop a full understanding and clear model of the amino structure of the JID region to base our modifications off of.

2. In silico Mutagenesis:
    - Perform virtual mutations on the JID and predict the exact AMINO (not mRNA) changes that would disrupt JAZ binding.
    - Use molecular dynamics simulations to assess the stability and behavior of mutated MYC2.
        i. Simulate the impact of mutations on MYC2's other functions (make sure MYC can still bind to)
3. mRNA Sequence Design:
    - Based on the optimal 3D model (or models if multiple), determine the corresponding mRNA sequences that need to be targeted.
    - Design CRISPR/Cas13 guide RNAs to target these specific mRNA ribonucleotides.
4. Error prevention and Predictive Analysis:
    - Use bioinformatics tools to predict potential off-target effects of the designed CRISPR/Cas13 system and work to minimize “off-targeting”.
    - Make sure CRISPR/cas13 system is pathogen inducible and becomes inactive once pathogen levels drop.
    - Begin wet lab phase!

(I’ll worry about phase 2 wet lab work when/if I finish phase 1 with conclusive findings, and I could do phase 1 as its own research paper and phase 2 as a validation of phase 1 as another paper). So in sum, I am first going to “protein-engineer”, then derive appropriate mRNA sequences/locations to mutate, then finally validate phase 1 predictions in vivo.

Is this project idea even feasible?

(If it is: how would I go about step 1 in phase 1 (would pyrosetta work)? There are multiple studies modeling MYC and predicting the structure of/modeling the JID region, and there is a general model of MYC2 in PDB, how would I use that past literature as a starting point?)