Designing a Fluorescence Based Mycobacterium Tuberculosis Death Reporter
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Abstract

Mycobacterium tuberculosis infection, commonly known as tuberculosis disease, is one of the most infectious and deadly diseases in the world, with an estimated 10 million people falling ill with tuberculosis and 1.5 million people dying each year from tuberculosis globally. One difficulty with studying tuberculosis is the large heterogeneity in infection for both bacterial and host responses. Current methods to study infection and bacterial death obscure the single cell heterogeneity of tuberculosis infection. To observe bacterial death at the single cell level, we developed a FlipGFP-based bacterial death reporter. As the bacterial membrane becomes permeabilized, cathepsins in the phagosome are able to cleave the cleavage site on the FlipGFP, allowing the FlipGFP to flip its orientation, permitting fluorescence. I developed a system with catalytically dead cathepsins, confirming that the cleavage of FlipGFP by active cathepsin was inducing any change in fluorescence. To optimize the reporter, after surveying the literature, I developed a suite of FlipGFPs with differing cleavage sites utilizing cathepsin B. I measured their fluorescence when exposed to host cathepsins, discovering a cleavage site with increased fluorescence compared to the original. I have validated and optimized this reporter which can be used to better understand bacterial death.

Background

• Bacterial death and host response in tuberculosis infection is heterogeneous and poorly understood.
• Current experimental methods to measure bacterial death utilize bulk timepoints, obscuring single cell heterogeneity.
• Host cathepsins are proteases which play a major role in bacterial infection response.
• Our reporter utilizes FlipGFP as a reporter of protease activity.
• GFP β-strands 10-11 are held apart by coiled coils, preventing association with GFP 1-9.
• Active protease of interest cleaves the cleavage site on the linker, allowing GFP 10-11 to assume its natural configuration.
• Cleaved GFP 10-11 reassembles with GFP 1-9, producing GFP fluorescence.

Design

FlipGFP reporter provides a tool to visualize permeabilization of cell membrane
• Express FlipGFP in Mycobacterium.
• Upon cell membrane permeabilization, FlipGFP becomes accessible to cathepsins in phagosome.
• By utilizing membrane permeabilization as a proxy for cell death, the FlipGFP reporter can visualize bacterial death inside the macrophage.

Results

Dead Cathepsin Exposure Provides Little Increase in Fluorescence Signal
Macrophage infection with Mycobacterium smegmatis confirms dead cathepsin provides little change in signal

References


Development of Novel Protease Cut Sites

Conclusions

• Dead cathepsin serves as a valuable negative control to study and improve the FlipGFP reporter.
• FlipGFP3 is cleaved by cathepsinB more efficiently when compared to the original cleavage site.
• FlipGFP3 may be a useful tool for studying Mycobacterium death in infection.

Future Works

• Further investigation of novel cleavage sites.
• Human macrophage infection with FlipGFP3 to measure signal in infection setting when exposed to host cathepsins.
• Fluorescence Microscopy of infection with novel cut sites.

Acknowledgements

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This sentence is a bit too long and so gets confusing. Also as a nitpick, you use the word "develop" a lot of times.

I think you should be more specific—what part of tuberculosis infection is heterogeneous and poorly understood? i.e. what part are you studying?

nitpick, but you use this phrase a lot!

I don’t know that this is true—cathepsin cleavage sites are very studied. Also, the point of building this reporter is not to study cleavage sites, so this isn’t the best thing to say here.

if you’re discussing moving to cathepsin B as a matter of optimization, it would be good to also briefly mention what you are optimizing from (cathepsin S) and why moving to cathepsin B is an optimization compared to that.

It’s generally good to put periods at the end of your bullet points, even if it’s not...
exactly a full sentence!

CRZ7 I think it’s better to just title this section “results”. I have validation and optimization on my poster because I’m showing data on the initial validation that the reporter works at all, and then showing data that improves upon that first test. Since your work starts sort of in the middle of a project, the data that you plan to show is not validation of the design, and it also is awkward to call it optimization since you don’t show data about what you’re optimizing from.

CRZ8 I would specify cathepsin B here

CRZ9 specifically single-cell heterogeneity. (there are other types of heterogeneity, like inter-donor, population-wide, etc.)

CRZ10 Font sizes here are a little awkward!

CRZ11 “scanned” is a bit colloquial, maybe say “reviewed” or “searched”

CRZ12 I don’t know if “novel” is the right word here, since
these are previously-reported upon cut sites. maybe just say "new"

Christine R Zheng.
2022-08-02T03:14:02.131

CRZ13 I think "analyzed" is not the right verb. maybe "tested"
Christine R Zheng.
2022-08-02T03:14:36.339

CRZ14 Sub-titles should be more descriptive! What do you want the viewer to conclude from this graph? Just say that as the subtitle
Christine R Zheng.
2022-08-02T03:16:04.658

CRZ15 What you are observing is not expression. Expression = amount of protein that gets translated. Since the reporter is always driven by hsp60 in every strain, expression is actually the same between all strains. Fluorescent signal is what is different
Christine R Zheng.
2022-08-02T03:18:09.954

CRZ16 Avoid using words like "normally". Instead, state what you mean by "normally", i.e. when expressed alone, in the absence of cathepsin B.
Christine R Zheng.
2022-08-02T03:18:41.033

CRZ17 You can be more conclusive! What does the increase in signal compared to the original cleavage site mean about FlipGFP3? It means that it responds to Cathepsin B activity, while the original does not
Christine R Zheng.
2022-08-02T03:21:14.533
What is the purpose of doing this infection?

Christine R Zheng,
2022-08-02 03:21:39.654