**Line 41: Please include some sort of introduction to the biofilm formation in *S. aureus* as well.**

We added some to the introduction in reference to biofilm (Line 64) and revised the paragraph as well.

**Line 75: Please include composition of both liquid and agar medium. Do you sterilize the medium before use? If yes how. Please include these details in the step as well.**

We add a new section of the protocol: Medium preparation (Line 82 to line 92). All compositions are included.

**Line 78: What volume?**

See revision, Line 97.

**Line 81: Please clarify are you plating in triplicate in one 96 well or using 3 96 well per time point.**

See revision, Line 103-Line 104

**Line 84: Does this lead to biofilm formation? Please provide reference if any?**

Yes, it does. To clarify, we cited our previous publication and another reference as well (Line 107).

**Also, do you perform the centrifugation step here?**

No, we didn’t. Since *S. aureus* forms biofilm at the bottom and sides of the plate and we aimed to measure ALP activity in biofilm, thus we needed to remove the suspended cells by aspiration.

**Line 86: How easy or difficult it is to decant by aspiration?**

It is fairly easy, although it is quite tedious. If you have a multi-channel pipet it takes less than 10 pipet pumps.

**You mention that ALP is secretory protein, then why do you decant the supernatant? One would assume that the protein would be found in the supernatant.**

Good point, in the current revision, we further emphasized that (see Line 34-Line 35, and Line 167) “its activity has been detected exclusively on the cell membrane”. This evidence was provided by Okabayashi, et al (ref # 7, pg 290).

**Line 88: How do you avoid the cell lysis?**

We convert G force to the respective rpm (8000 rpm), cells have been viable after this speed and we have not seen issues in ALP activity. Cell lysis should not pose a problem with this rotation or force.

**Line 91: This assay is showing the measurement with different time point of incubation with pNPP. So basically, the time it takes with NPP to develop color.**

**However, this does not show how ALP measurement is related to biofilm formation. Need some sort of result to show the same. Maybe different time point of biofilm and then the assay to go in alignment with the manuscript.**

Data suggesting a relationship between ALP activity and biofilm has been shown in our previous study. We added a note and a reference to previous data.

**Line 110: There should be a result figure to show that indeed you are checking the activity in biofilms**

**Then the figure presented here which suggest different incubation time with the substrate. Also needed is some sort of control.**

**Then one more which suggest that you are able to differentiate ALP production in biofilms generated at different time by same species or either by different species.**

In our previous publication, we already established that ALP activity is elevated in biofilm compared to its suspension part, thus, ALP activity might be a molecular marker for biofilm formation or manipulation. Whether this elevated activity caused the biofilm formation or not needs further investigation.

As a follow up for this project (also as editor suggested), we will be studying the time course of ALP activity corresponding to the extent of biofilm formation. This is an ongoing project, we are not ready to publish the data yet.

Since this manuscript is to measure ALP activity in biofilm, our current data should be sufficient at this point.

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To avoid copy right issue, we produced a new figure based on our result, this figure has not been published.

**Line 123:**

**As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:**

**a) Critical steps within the protocol**

**b) Any modifications and troubleshooting of the technique**

**c) Any limitations of the technique**

**d) The significance with respect to existing methods**

**e) Any future applications of the technique**

We made some minor revisions in the “Discussion”. For critical step, see Line 152. For modifications, see Line 159 to Line 161. For limitations, see Line 172. For future applications, see Line 175 to Line 177.