# Review of: "Light/dark and temperature cycling modulate metabolic electron flow in Pseudomonas aeruginosa biofilms"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

## Review

### Summary

This paper manuscript describes a very well thought study on the effect of light/dark and temperature cycling on P14*P. aeruginosa* biofilm growth and metabolism. The paper manuscript connects the missing link between light exposure at different rhythmic patterns and biofilm responses to resulting temperature changes. The authors were able to identify key molecular players that are sensitive to these physical changes and alter metabolic electron flow in *P. aeruginosa* biofilm.

### Recommendation

I recommend this paper manuscript to be published, as it is a well thought, decently planned study that starts from morphological observation to chemical quantification, and from kinetic measurement to molecular identification, in a logical, top-down approach. This paper manuscript provides an opening and insights to researchers interested in this field, and potentially expansion into many other physical factors that may have similar or different effect on bacterial biofilm.

### Correction

I would recommend authors to answer my questions and provide experimental results as needed. I do not have any request on further correction or revision.

### Questions

1. Have you looked into how the effect of burst vs. prolonged exposure of*P. aeruginosa* biofilm to light or temperature? For example, if you expose one set of biofilm samples to light 5 minutes on and 5 minutes off through the whole entire day time (i.e., 12 hours), and expose another set of biofilm samples to continuous light through the whole entire day time (e.g., 12 hours) , could there be different effect on bacterial metabolism?
2. In Figure 2, it is interesting to observe that the distance between dark rings seems to increase as the duration of exposure increases. I sense that there might be a correlation between 2 factors. One could explain it could simply be because biofilm will show more repeated dark rings at a shorter repeated exposure period assuming biofilm growth is

at a constant rate (but different rates between exposed and non-exposed). If this is truly the case, then one should be able to build a calibration curve with a linear regression, assuming the distance between dark rings correlates positively (potentially linearly) with light exposure time. Could you please provide some data and possibly a calibration curve? It should be relatively simple to do on your already existing data, and maybe add 2 more time points (e.g., 3 hours and 18 hours).

3. In Figure 2, the intensity of outermost ring seems to increase as the duration of exposure increases, but saturate between 12 hours and 24 hours. It would be interesting to see what happens between 6 hours and 12 hours, and if this shorter exposure has any effect and possibly provides any insight on *P. aeruginosa* metabolism as a function of rhythmic exposure time. Could you provide some data on this aspect?
4. Is this light/temperature sensitive feature specific to P14 *P. aeruginosa* biofilm, or also observed in other *P. aeruginosa* (e.g., PAO1) biofilm? What about other non-phototrophic and phototrophic bacterial biofilm? Is this specific to Gram negative bacteria, or can this be also true for Gram positive bacterial biofilm (e.g., *S. aureus*)?
5. Does light/temperature sensitive feature (not including light wavelength results in your study) affect bacterial (biofilm) growth, horizontally and/or vertically? Does it affect the size of spread (e.g., diameter of ring as a function of light exposure time)? Does it affect the thickness of biofilm (e.g., thickness of biofilm (centre vs. middle peripheral vs. outermost peripheral) as a function of light exposure time)? Does this feature have localized effect (e.g., if exposing only a part of biofilm, would rings develop very differently and would this affect biofilm growth around this exposed region?)? What about kinetics effect?
6. Does light/temperature sensitive feature affect the morphology of biofilm? Some *P. aeruginosa* spread in ring-like structures, and some spread as pseudopod- or dendritic-like structures. Could you provide some insights on this?
7. In Figure 3, it is great to see that you have done kinetics work on this. This helps explaining some biochemical aspects of this study. On your existing data, could you provide some insights on if TTC reduction is a 0, 1st, or 2nd order reaction? What about TCC half life, initial velocity (Vi), and maximum velocity (Vmax)? Are these values different between exposed and non-exposed biofilm, and different by what factor (e.g., 2-fold, 4-fold, etc.)?
8. You stated that P14 *P. aeruginosa* biofilm retains this property after light exposure. Is this property generalized across most *P. aeruginosa* strains? Similarly, across other Gram positive bacteria? Is there any bacterial strain that might lose instead of retaining this property?
9. This is another biochemistry question from Question 7 (and the last question). Have you done these kinetics experiments at 37oC (i.e., physiological temperature)? Could you provide some data on this, as biofilm growth can be very different at room temperature vs. physiological temperature?

## Conclusion

After authors answer my questions and provide experimental results, I recommend publishing this paper manuscript as it is (including some experimental results requested).