

# Characterizing hypovesiculating mutants in *Porphyromonas gingivalis*



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## Introduction

- *Porphyromonas gingivalis* (Pg) is a Gram-negative bacterium proven to be a major contributor to periodontitis, or gum disease.
- The outer membrane (OM) of *P. gingivalis* is a phospholipid bilayer with various associated structures on the outer leaflet. Outer membrane vesicles (OMVs) bud off of the OM. The mechanisms responsible for OMV formation are not fully understood in Pg or other Gram negative bacteria.
- OMVs are an important mediator of interactions between Pg and the extracellular environment. Lipopolysaccharide (LPS) is an abundant component of the OMVs.
- LPS is a major component of the OM made up of lipid A, the core, and the O-antigen. Two forms of LPS exist. The difference in between them is in the O antigen component of the LPS structure.

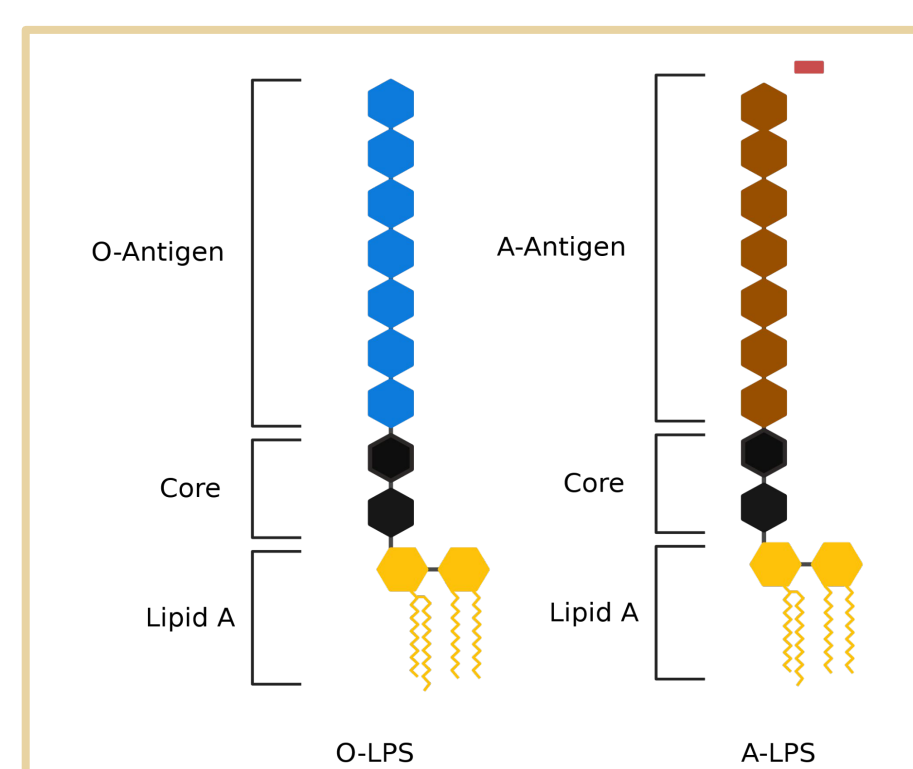


Figure 1 - The components of the lipopolysaccharide structure - O/A-antigen (blue/brown), core (black) and lipid A (orange).

- OMVs are also expected to interact with the host's immune system through the TLR4 receptor, contributing to the inflammation associated with periodontitis.

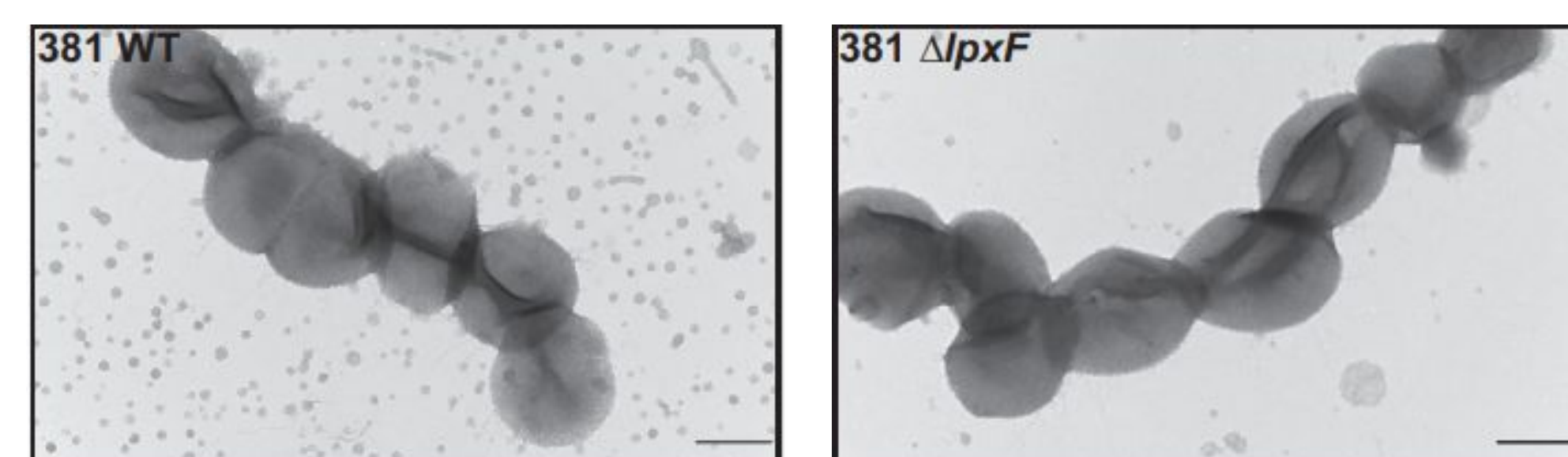


Figure 2 - Transmission electron microscopy images of WT and  $\Delta lpxF$  in genetic background 381. WT creates an abundance of vesicles whereas the deletion mutant,  $\Delta lpxF$ , does not. Vesicle production is likely impaired due to the retention of the C4 phosphate group. - adapted from Alaei et al. 2024

## Preliminary Data

- Our lab previously observed that deletion mutant  $\Delta lpxF$  created a significant reduction in OMV production across three different *P. gingivalis* strains.
- Deletion mutant  $\Delta lpxF$  is known to induce increased levels of TLR4 stimulation on host cells.
- Publications from outside our lab have found similar phenotypic results after investigating the linkage between OMVs and biofilm production/aggregation patterns in other deletion mutants.
- $\Delta wbpB$  is a deletion mutant that interferes with the synthesis of A-LPS structures that are typically present on the OM.

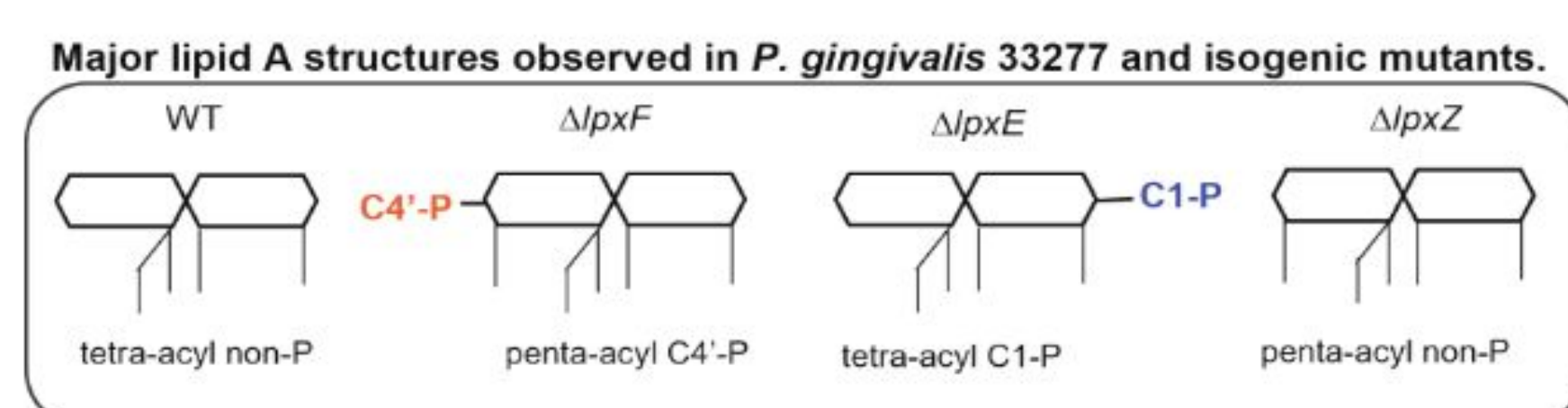
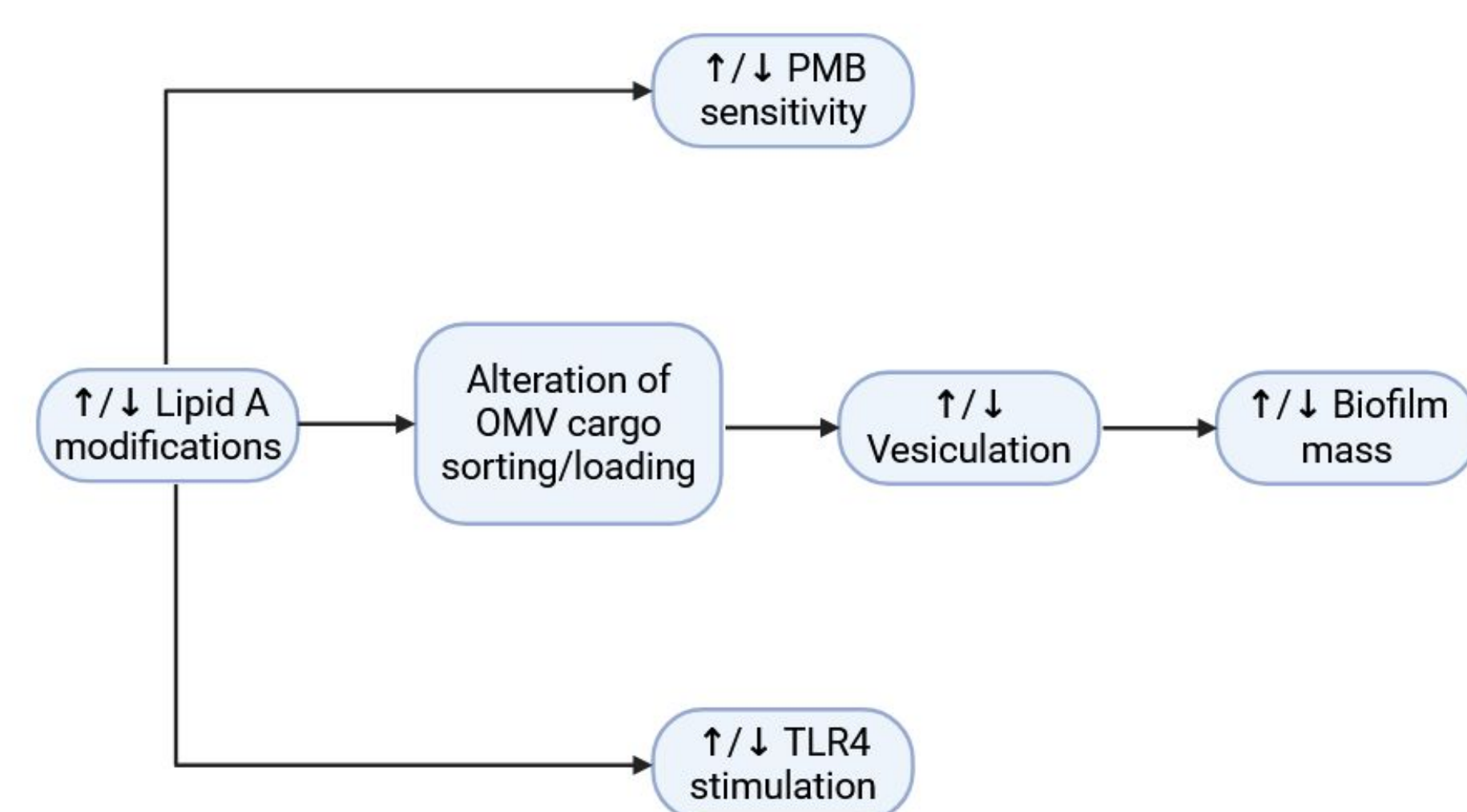


Figure 3 - Deletion of lipid A modifying enzymes results in altered lipid A structures in *P. gingivalis*. Our lab previously discovered that the presence of all C4'-lipid A significantly reduces OMV production. - adapted from Alaei et al. 2024

## Hypothesis



## Research Goals

1. Acquire transmission electron microscopy (TEM) images for the new mutant
2. Compare polymyxin B sensitivity to assess lipid A modifications
3. Utilize an ELISA to measure TLR4 stimulation by LPS on human monocytes
4. Quantify total biomass for biofilms with a crystal violet assay

## Methods and Results

### Transmission Electron

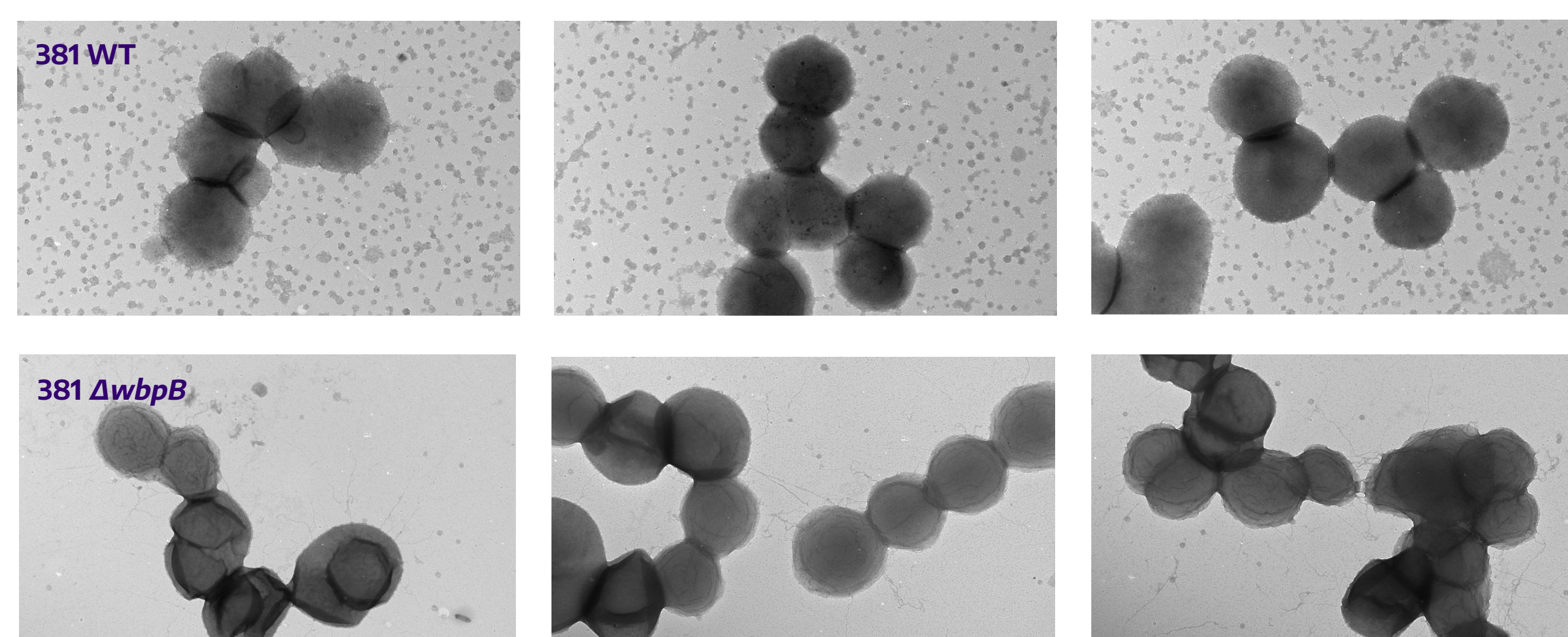
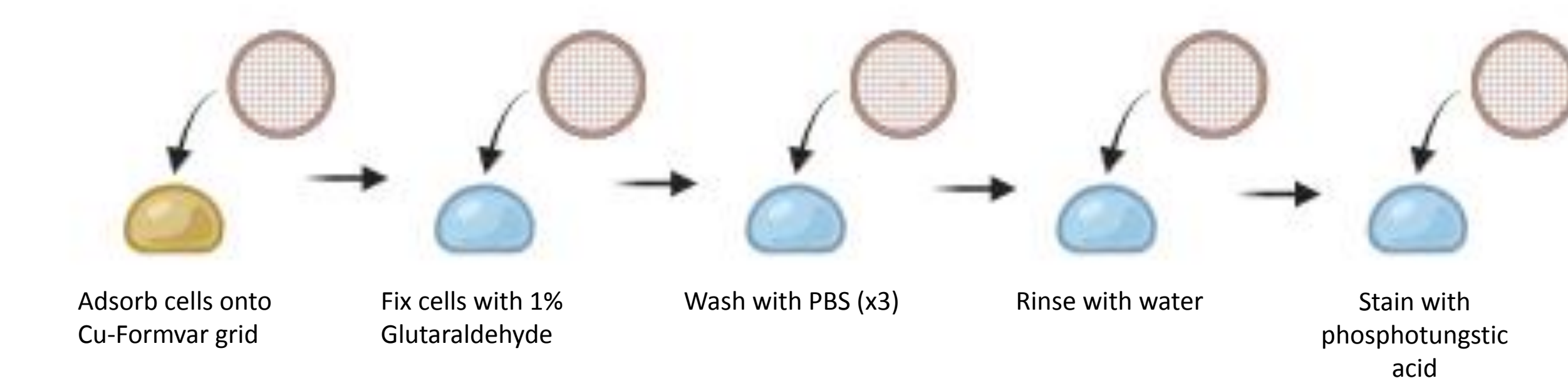


Figure 4 - Methods for the preparation of TEM Cu-Formvar grids and the resulting images. Subcultured Pg was adhered onto Cu-Formvar grids and imaged on a transmission electron microscope. Images were captured at 130000X total magnification. 381  $\Delta wbpB$  displays a dramatic decrease in vesicle production akin to  $\Delta lpxF$ .

## Polymyxin B sensitivity + Total biomass via crystal violet

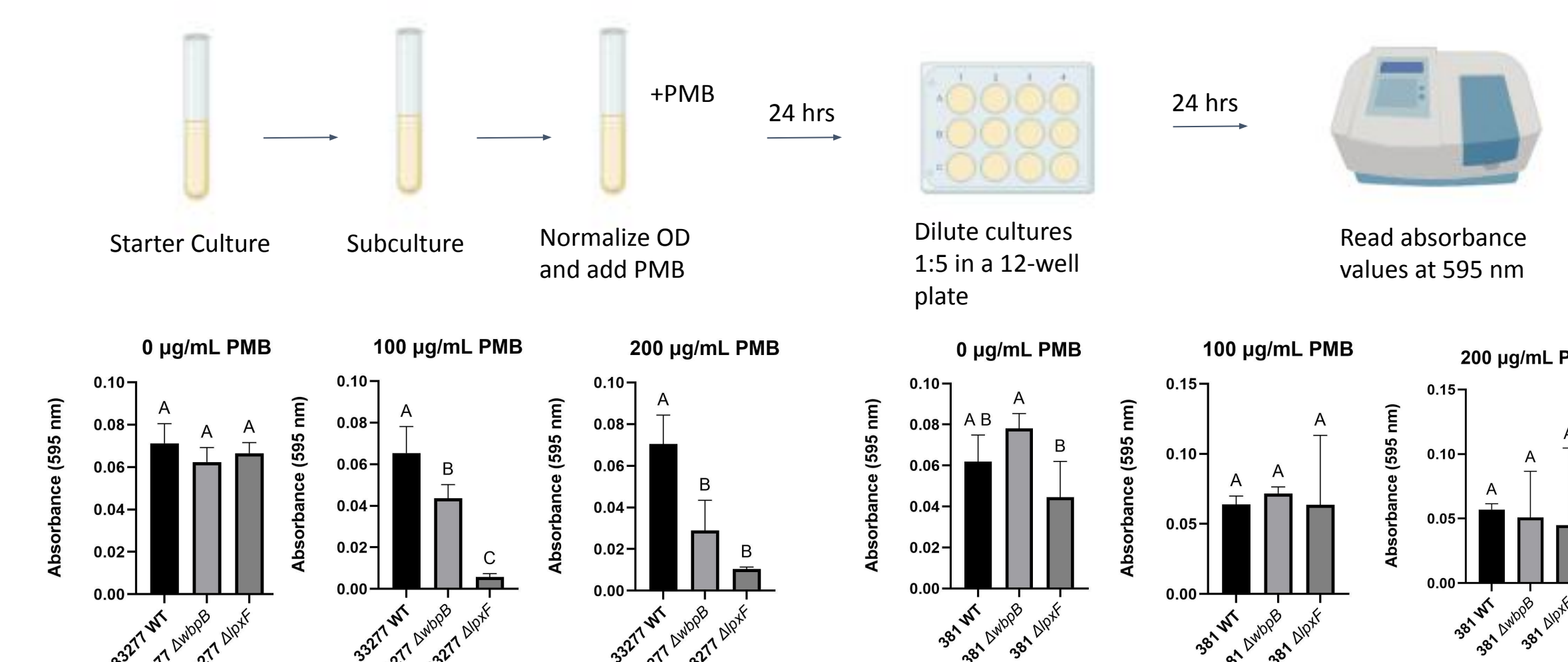


Figure 5 - PMB sensitivity assay methodology and results. PMB sensitivity in 381 background does not follow the expected sensitivity pattern. 381  $\Delta lpxF$  does not display significant sensitivity compared to WT, indicating a possible inconsistency with experimentation. 33277 strains follow the a sensitivity pattern that corresponds to the vesiculation gradient observed on TEM images previously.

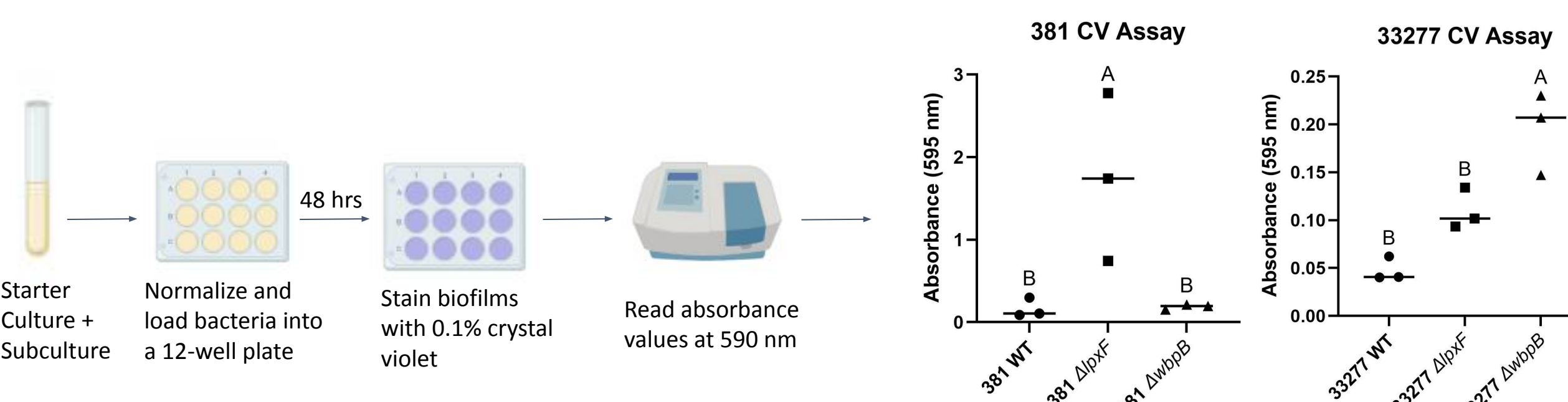


Figure 6 - CV assay methodology and results. Total biomass quantification for 33277 strains do not follow the expected pattern based on vesiculation observed in TEM images. This mutant is significant from both WT and  $\Delta lpxF$ . In 381,  $\Delta wbpB$  is not significant from WT. These experimental results support the fact that while there is likely a lot of overlap, OMV production is not explicitly defined by lipid A structure.

## Enzyme Linked Immunosorbent Assay

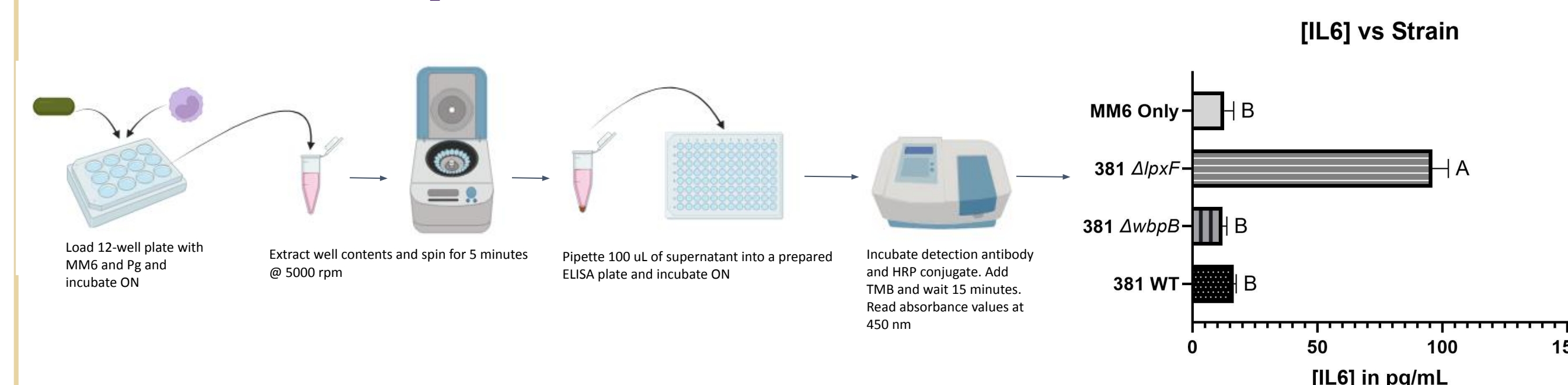


Figure 7 - MM6 exposure and subsequent ELISA results. Human monocytes were exposed to three different strains of Pg for 16 hours. The whole cell cultures were then spun down and the supernatant was used for antigen capture for the ELISA. The  $\Delta lpxF$  mutant functions as a positive control. Deletion mutant  $\Delta wbpB$  was not significantly different from WT.

## Implications and Future Directions

- Immediate first steps are to repeat the ELISA with the same strains to confirm results and possibly adding another polysaccharide mutant into the mix, affecting O-LPS rather than A-LPS.
- We will continue to investigate mechanistic convergence in OMV production pathways between other deletion mutants (e.g.  $\Delta relA$ ,  $\Delta rshB$ ,  $\Delta lptO$ ) and  $\Delta lpxF$ .
- After fully describing the mechanism for OMV production we can start precisely manipulating them for clinical and research applications (e.g. block production, vaccine delivery with other OMV producing species).

## References & Acknowledgments

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