



# SPATIAL ORGANISATION OF ENZYMES IN THE BIOSYNTHETIC LIMONENE PRODUCTION PATHWAY IN ESHERICHIA COLI

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Tiffany Chau, Thomas Lautier, Poh Chueh Loo, Zhang Congqiang, Cédric Y Montanier, et al.. SPATIAL ORGANISATION OF ENZYMES IN THE BIOSYNTHETIC LIMONENE PRODUCTION PATHWAY IN ESHERICHIA COLI. Enzyme Engineering XXVII, Oct 2023, Singaour, Singapore. hal-04225784

**HAL Id: hal-04225784**

**<https://hal.science/hal-04225784>**

Submitted on 3 Oct 2023

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# Spatial organisation of enzymes in the biosynthetic limonene production pathway in *Escherichia coli*

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## INTRODUCTION

Limonene has a wide range of applications, including its use in food, cosmetics, pharmaceuticals, or chemistry.

The mevalonate pathway (MVA) was engineered into *Escherichia coli* to produce limonene from glucose<sup>[1]</sup>. The final two steps of the pathway, the condensation of DMAPP and IPP to GPP and the cyclisation of GPP to limonene<sup>[2]</sup>, are thought to be **rate-limiting**, as production of monoterpenes has remained lower compared the production of diterpenes, sesquiterpenes, and tetraterpene<sup>[1]</sup>.

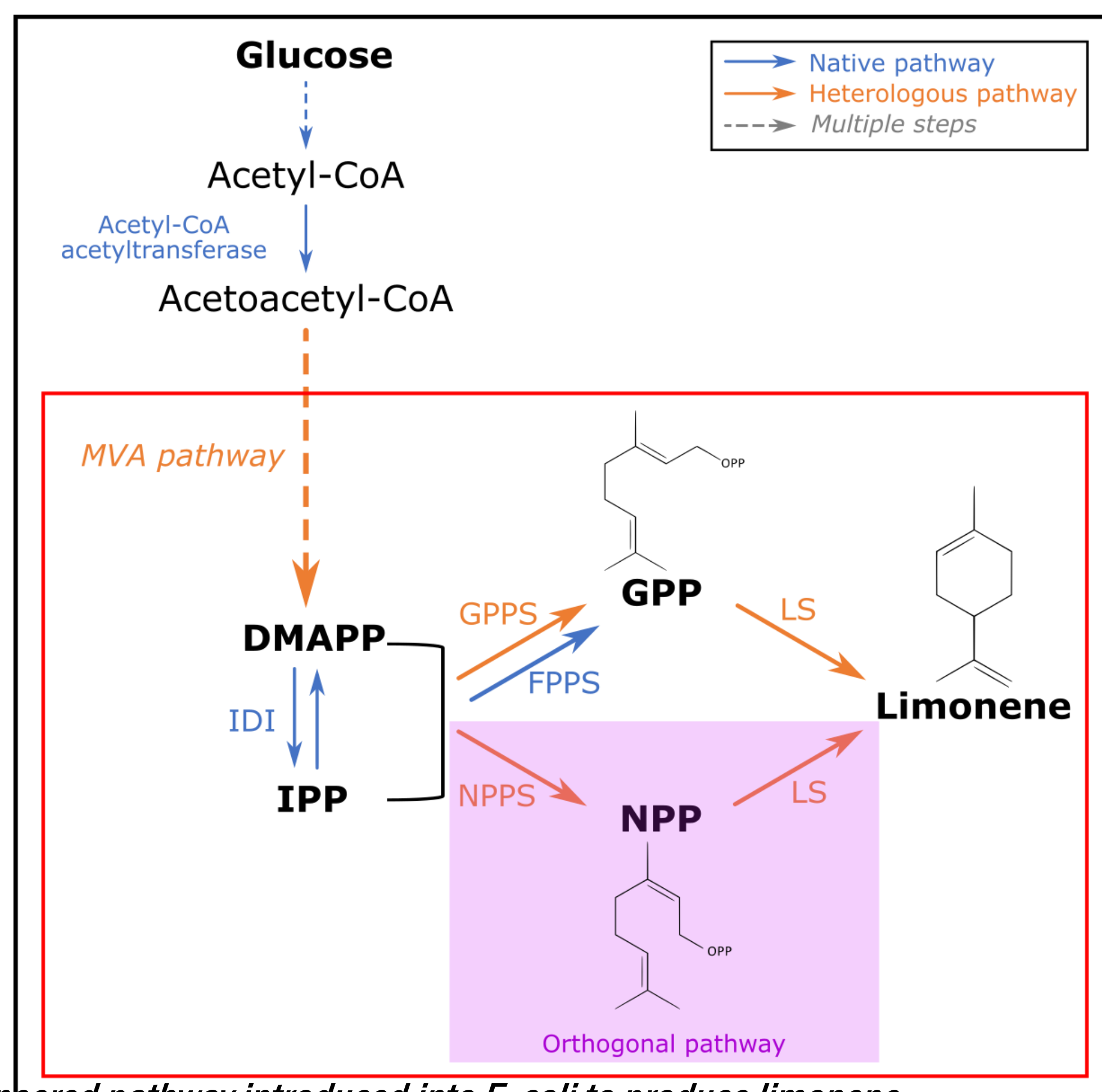


Figure 1. Engineered pathway introduced into *E. coli* to produce limonene.

Inspired by examples from natural enzymes such as tryptophan synthase, carbamoyl phosphate synthase<sup>[3]</sup>, the spatial organization of enzymes is hypothesized to improve pathway flux. This study aims to **increase limonene production** in *E. coli* by playing with the spatial organisation of enzymes.

## OBJECTIVES

**Increase limonene production** using translational and post-translational methods of assembling enzymes.

Experiment with **different homologues**, including utilising an orthogonal pathway.

## PAIR 1: GPPS + LS

Attempts to increase limonene titres with **GPPS** and **LS** homologues from Alonso-Gutierrez *et al.*<sup>[1]</sup> by translational fusions and post-translational fusions were both unsuccessful.

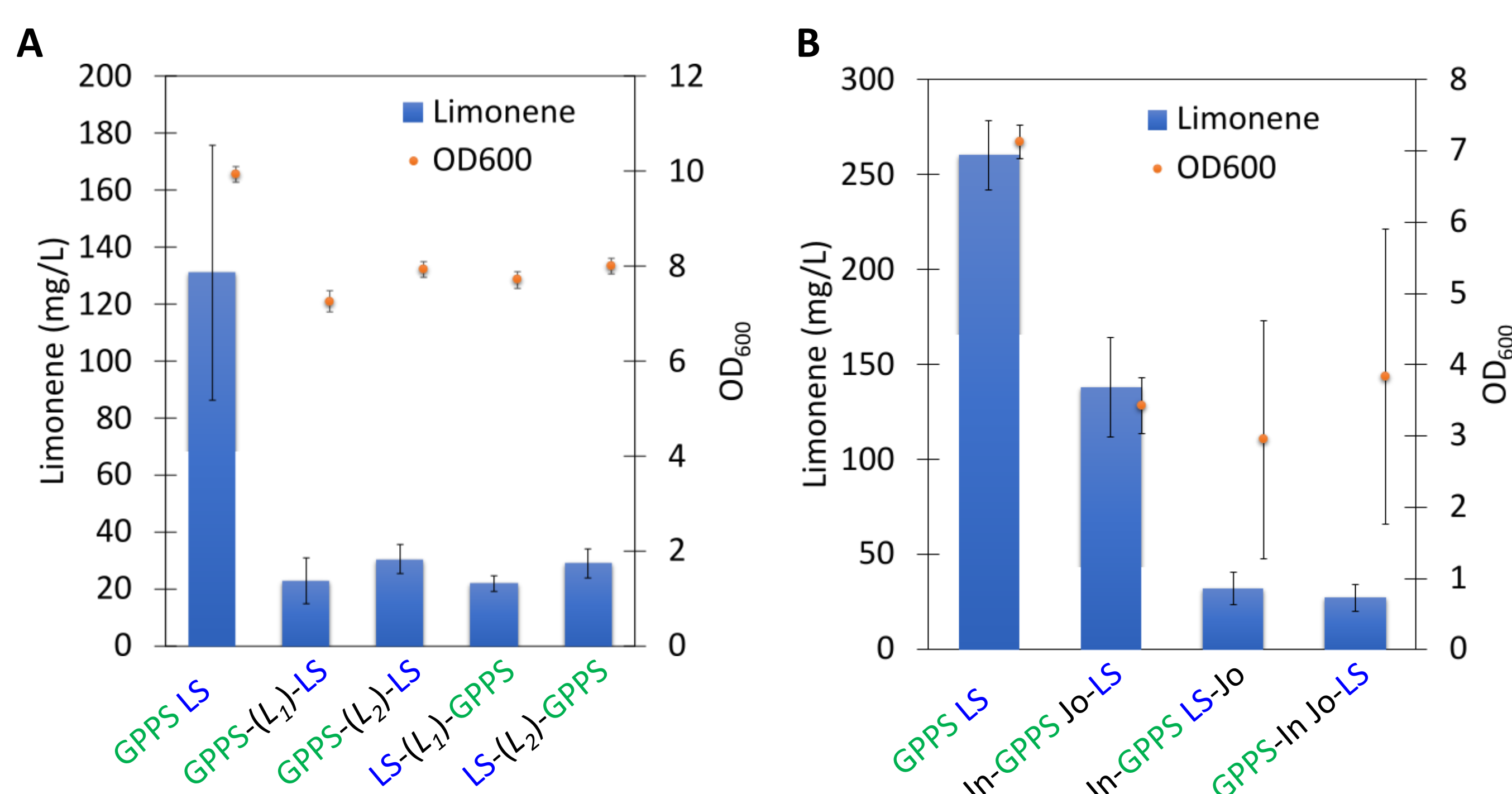


Figure 2. Limonene production in *E. coli* with (A) translational fusions and (B) post-translational fusions.

## PAIR 2: FPPS + LS

Cheah *et al.* increased limonene titres in *S. cerevisiae* with a **translational fusion of an FPPS mutant and LS**<sup>[4]</sup>. However, when we experimented with these homologues, the enzymes were not very active in *E. coli*, where the free enzymes produced only marginally higher limonene compared to the non-induced negative control.

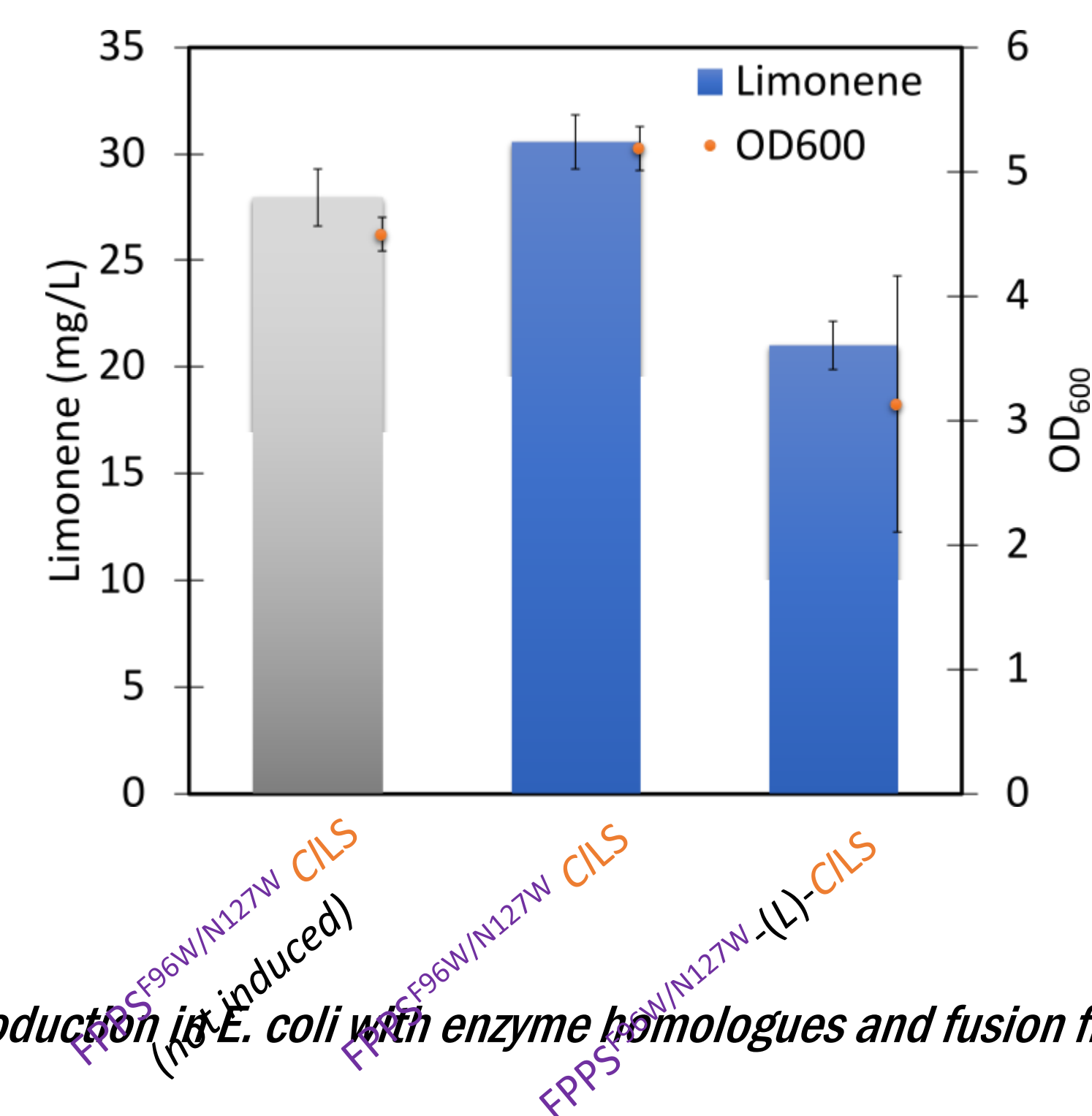


Figure 3. Limonene production in *E. coli* with enzyme homologues and fusion from Cheah *et al.*<sup>[4]</sup>.

## PAIR 3: NPPS + LS

We next experimented with **NPPS** and another **LS** homologue fusion, which had shown to increase limonene titres compared to the free enzymes<sup>[5]</sup>. While the referenced study was again done in *S. cerevisiae*, these homologues were active in *E. coli*, and the fusion achieved higher limonene titres than the free enzymes.

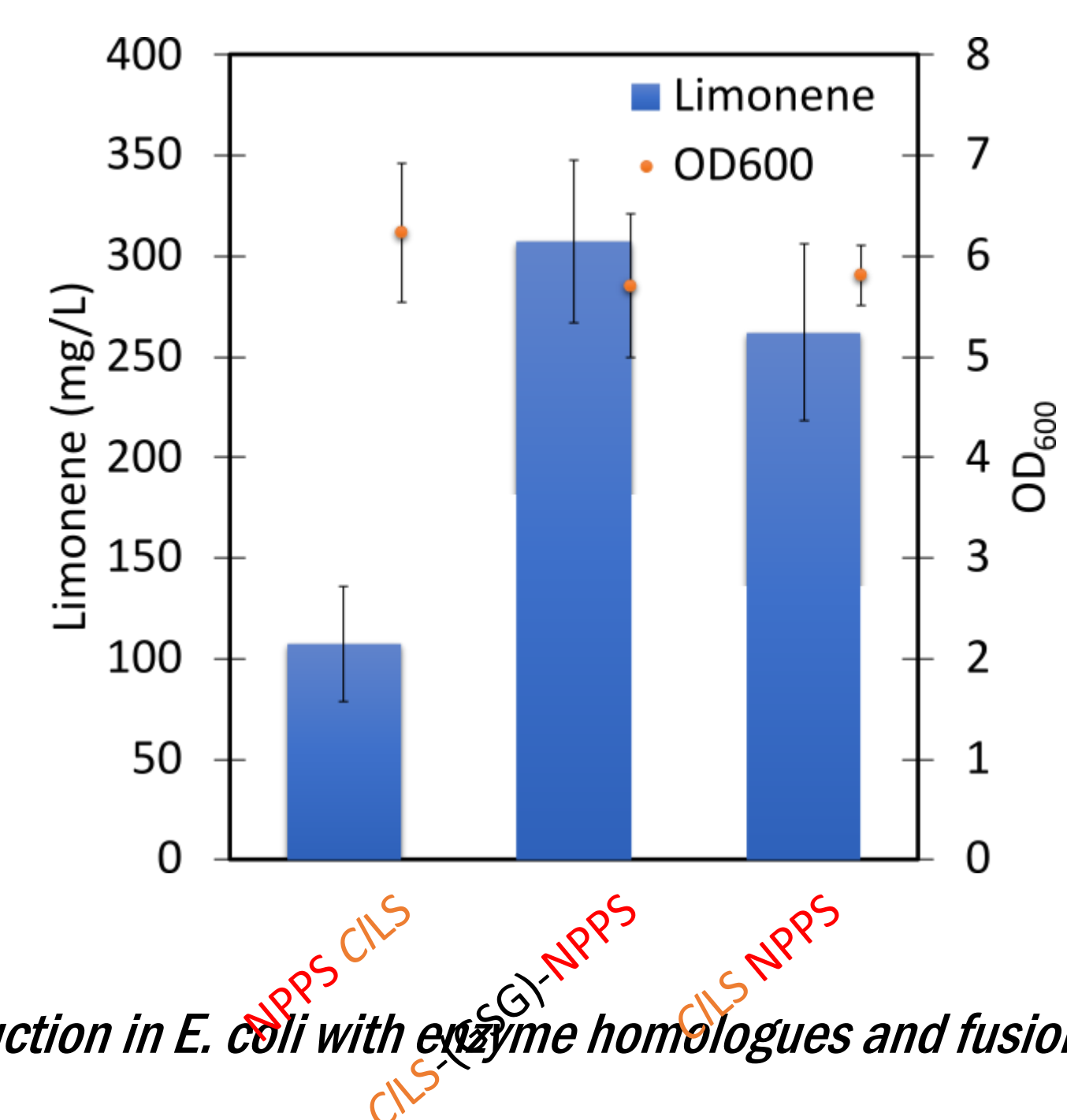


Figure 4. Limonene production in *E. coli* with enzyme homologues and fusion from Hu *et al.*<sup>[5]</sup>.

## CONCLUSION

We show that a **translational enzyme fusion achieves higher limonene production compared to free enzymes**, as previously reported<sup>[5]</sup>, albeit in *E. coli*. **Further work with post-translational assembly** will be carried out to investigate if post-translational assembly of these enzyme homologues are able to increase limonene production.

## REFERENCES

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## ACKNOWLEDGEMENTS

This work is funded by the EcoCTs project with the support of the National Research Foundation, Prime Minister's Office, Singapore under its Campus for Research Excellence and Technological Enterprise (CREATE) programme (NRF2019-THE001-0007).