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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>.

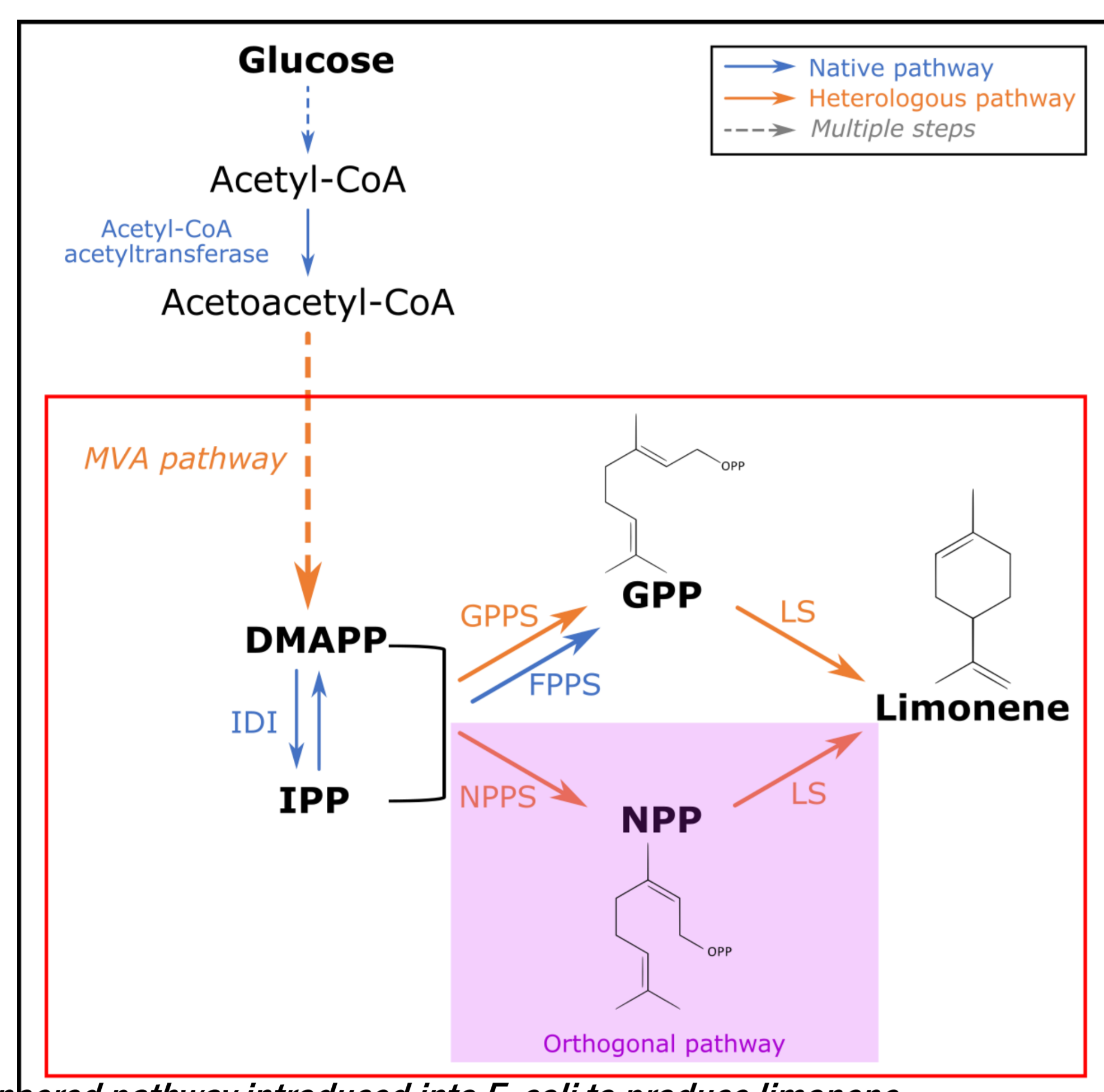


Figure 1. Engineered pathway introduced into *E. coli* to produce limonene.

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.

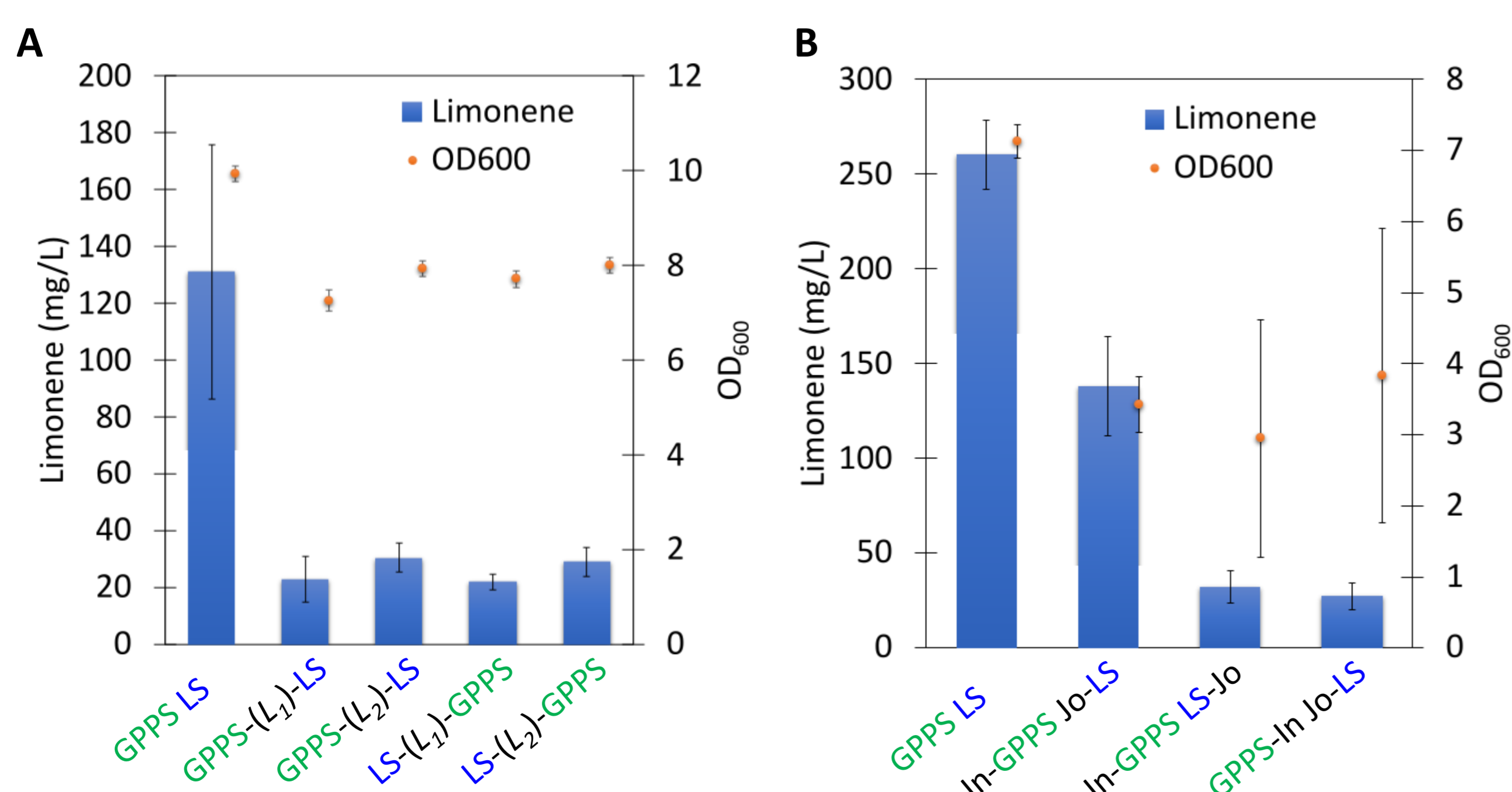


Figure 2. Limonene production in *E. coli* with (A) translational fusions and (B) post-translational fusions.

## 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.

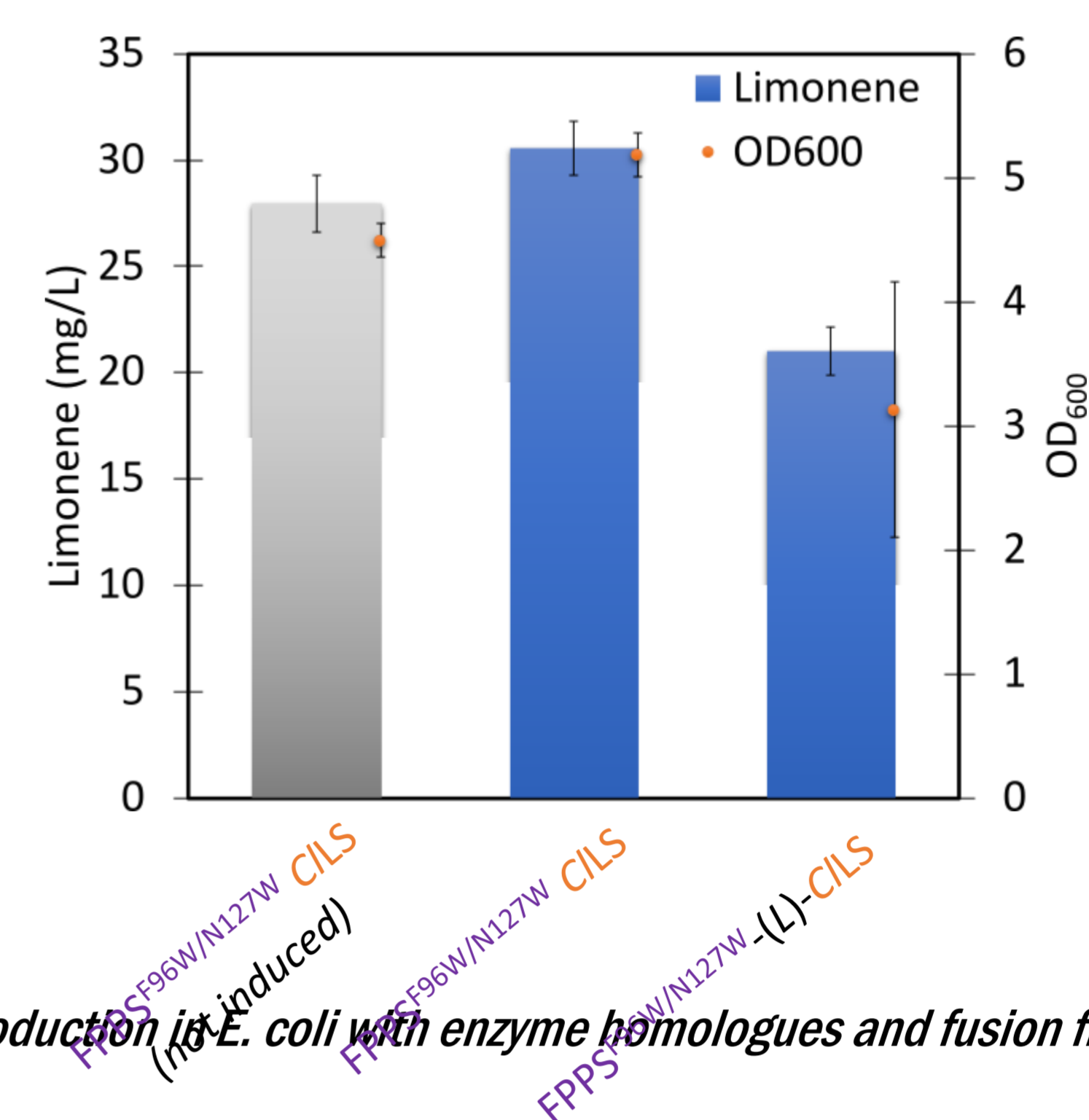


Figure 3. Limonene production in *E. coli* with enzyme homologues and fusion from Cheah *et al.*<sup>[4]</sup>.

## 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.

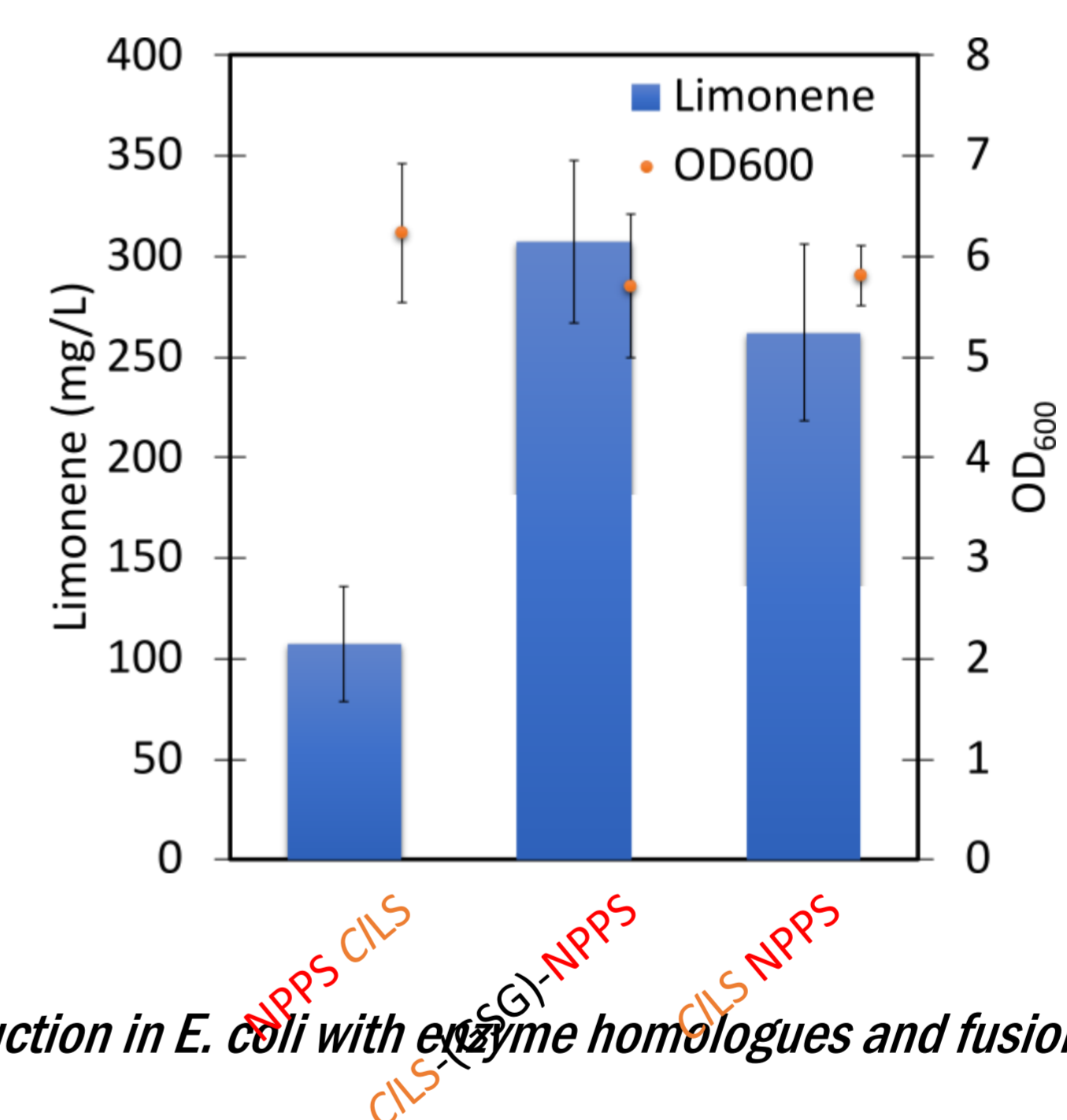


Figure 4. Limonene production in *E. coli* with enzyme homologues and fusion from Hu *et al.*<sup>[5]</sup>.

## 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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