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Post-transcriptional association of proteins to study spatial organisation within multi-enzyme complexes

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Background

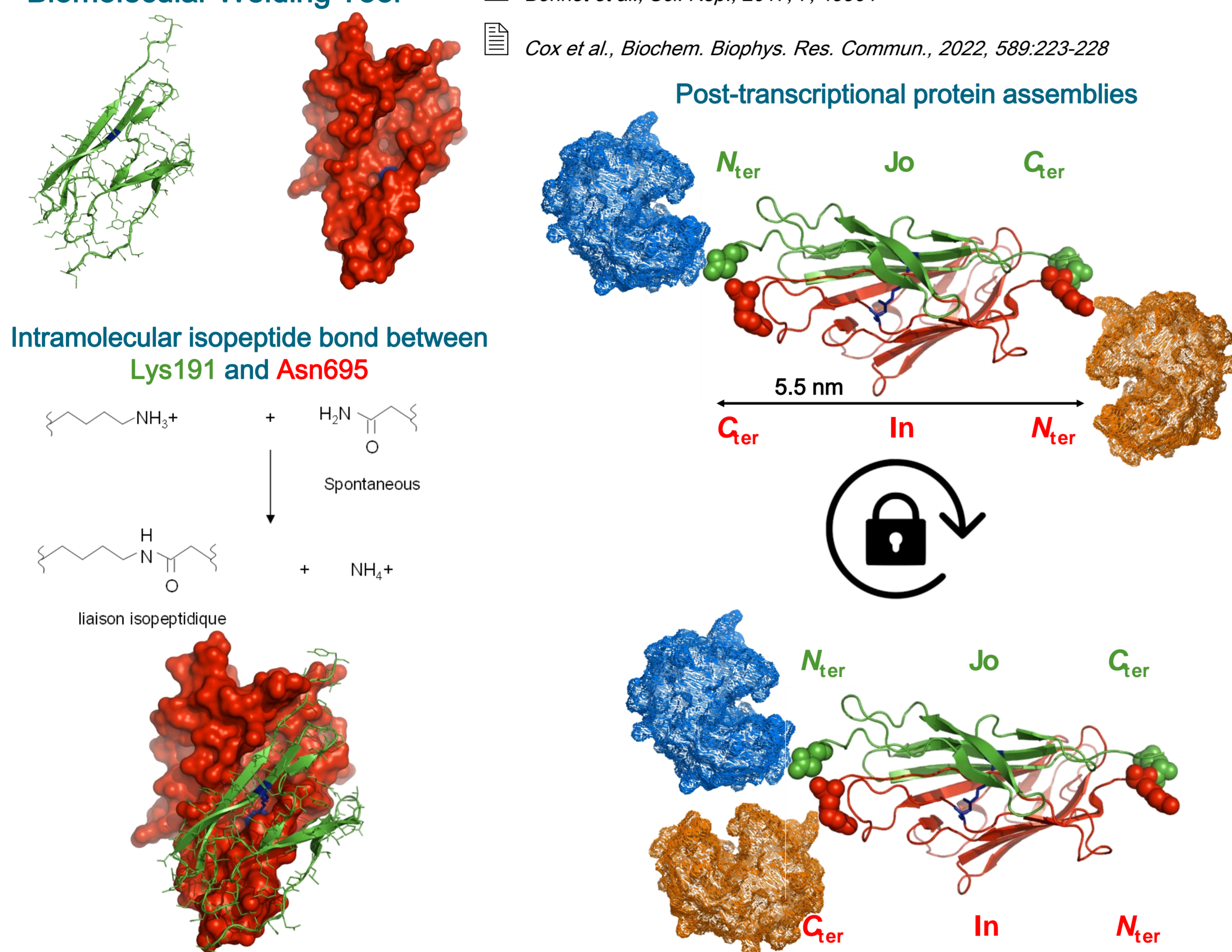
To catalyse chemical reactions, nature has evolved enzymatic cascades. These multistep reactions in living cells are often performed by multi-enzyme complexes to maintain high local concentrations of intermediates to enhance reaction rates, called substrate channelling. Based on the fact that natural proteins or nucleic acid-protein interactions can be used as scaffolds for the construction of functional multi-enzyme complexes, artificial scaffolds have been developed for the construction of multi-enzyme complexes which carry out multi-step enzymatic catalysis processes. However, none of them have systematically investigated the spatial organisation of the enzymes and its effect on the product(s) released. We are investigating this question using the Molecular Welding Tool consisting of two small proteins, Jo and In, which spontaneously form an intramolecular isopeptide bond and, incidentally, provide an original means of orienting enzymes. We exemplified our strategy inspired by two different type of multi-enzymatic organizations; the plant cell wall degrading enzymes complexes known as cellulosome and a heterologous pathway introduced into *Escherichia coli* to produce limonene from glucose.

Results

1

Biomolecular Welding Tool

Bonnet et al., *Sci. Rep.*, 2017, 7, 43564
Cox et al., *Biochem. Biophys. Res. Commun.*, 2022, 589:223-228



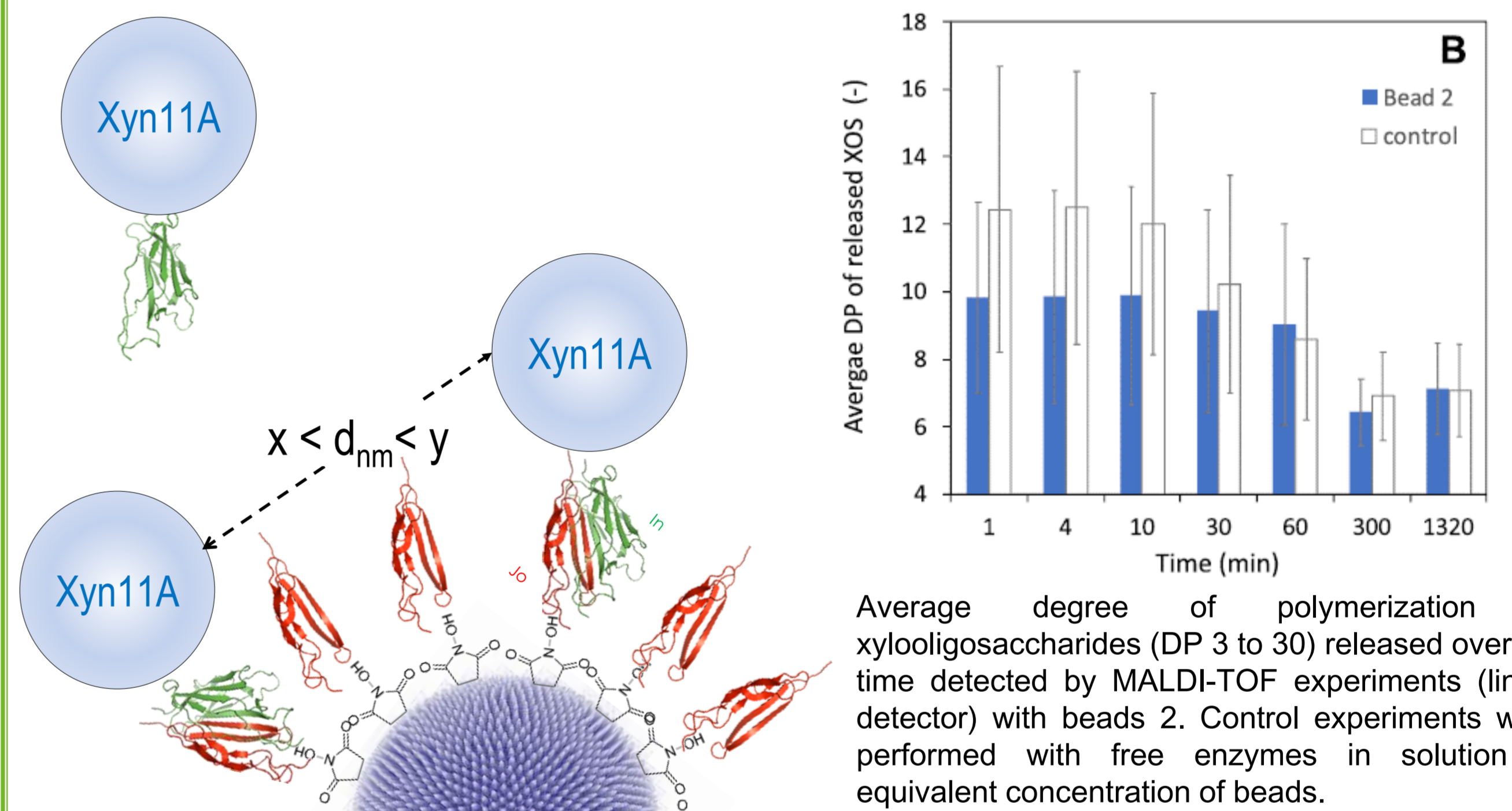
2

Precise control of the distance between immobilized enzymes using Jo and In recombinant proteins

Montanier et al., *Sci. Rep.*, 2019, 9,5763

| | In/NpXyn11A | Beads 0 | Beads 2 | Beads 4 | Beads 5 |
|---|------------------------|---------------------------|--------------------------|--------------------------|--------------------------|
| in solution | | 21.2 nmol.m ⁻² | 3.5 nmol.m ⁻² | 1.1 nmol.m ⁻² | 0.5 nmol.m ⁻² |
| K_m (mg.ml ⁻¹) | 1.8 ± 0.7 | 3.6 ± 0.5 | 3.6 ± 0.5 | 2.6 | 2.1 |
| k_{cat} (min ⁻¹) | 46.1 × 10 ³ | 8.9 × 10 ³ | 22.4 × 10 ³ | 43.5 × 10 ³ | 55.1 × 10 ³ |
| k_{cat}/K_m (min ⁻¹ .mg ⁻¹ .ml) | 25.6 × 10 ³ | 2.5 × 10 ³ | 6.15 × 10 ³ | 16.5 × 10 ³ | 26.2 × 10 ³ |

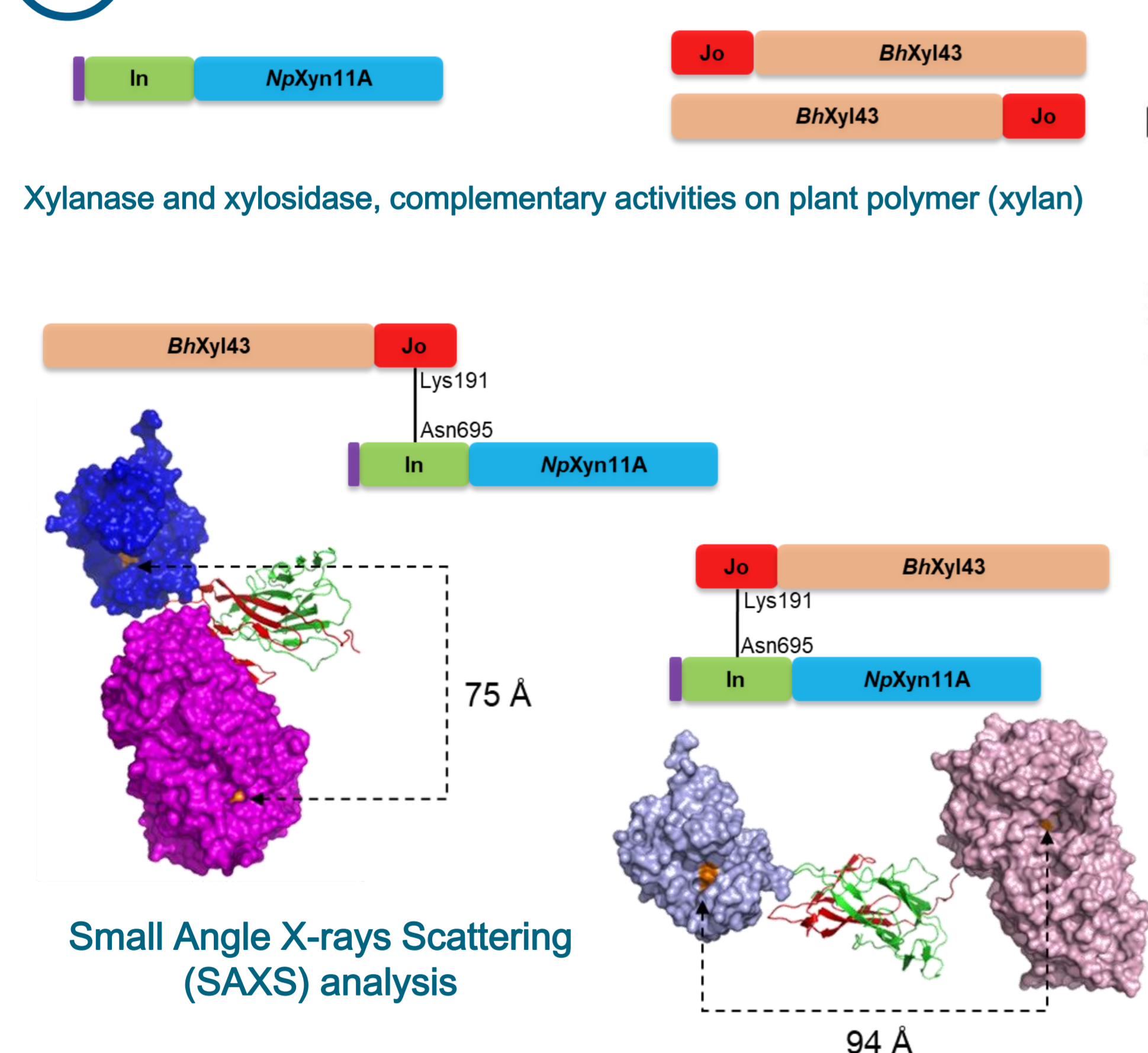
Kinetic parameters using beechwood xylan as long chain polymer



3

Chimeric GHs and spatial geometry

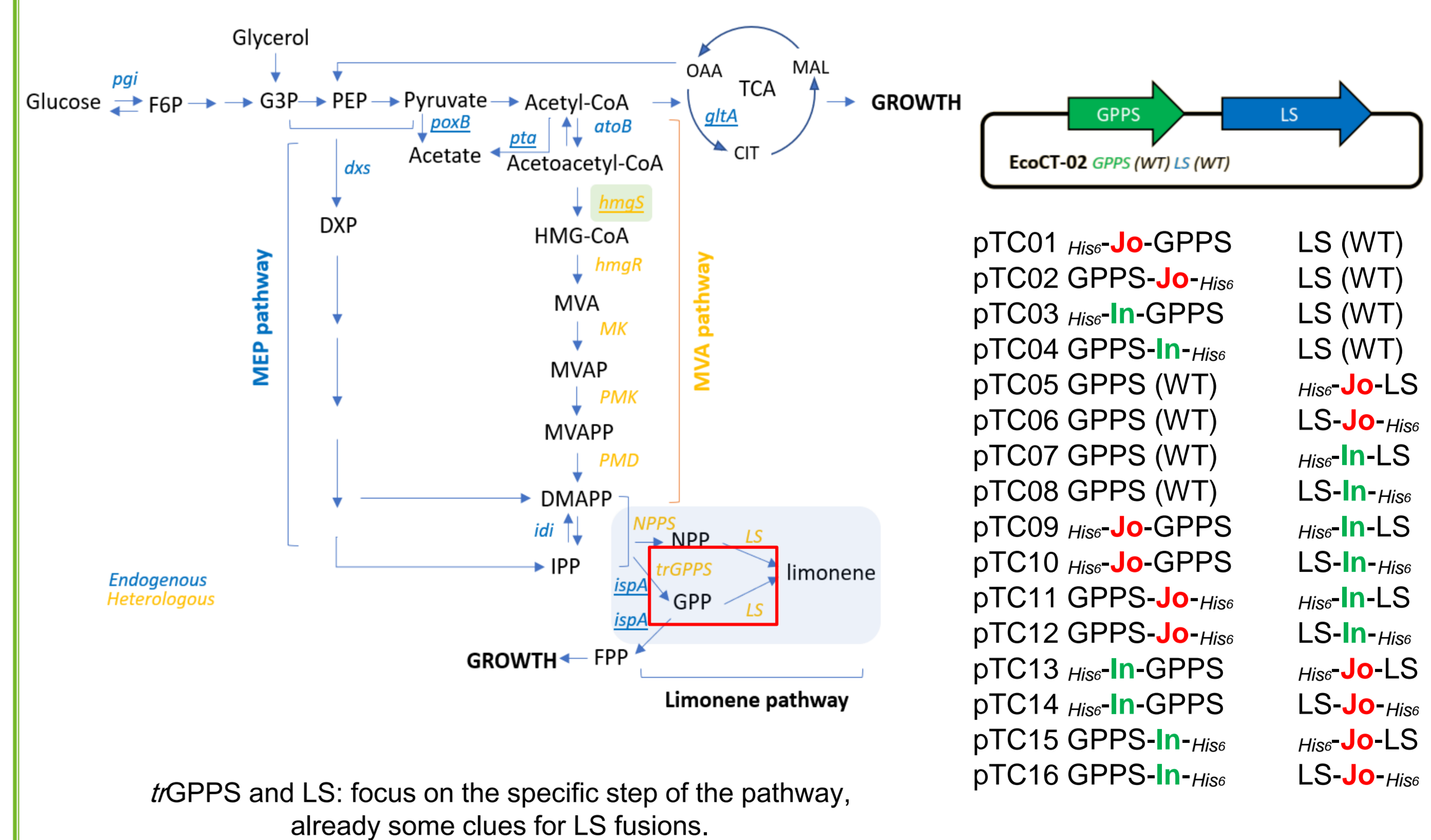
Enjalbert et al., *Int. J. Mol. Sci.*, 2020, 21(12), 4360
Badruna et al., *New Biotechnol.*, 2021, 65:31-41



4

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

Chau et al., unpublished data



Conclusion

We have shown that the BMW tool, through a spontaneous covalent association between Jo and In, represents an innovative approach to associating enzymes of interest in a controlled spatial organisation. Our main objective is to address the spatial proximity of enzymes involved in an enzymatic cascade and their relationship to their substrate. By analysing our various complexes using robust biophysical approaches, we aim to study the close relationship between an enzymatic complex and its substrate as closely as possible to what happens in vivo.