Making new PALs: A Directed Evolution Platform for Rapid Peptide Asparaginyl Ligase Engineering

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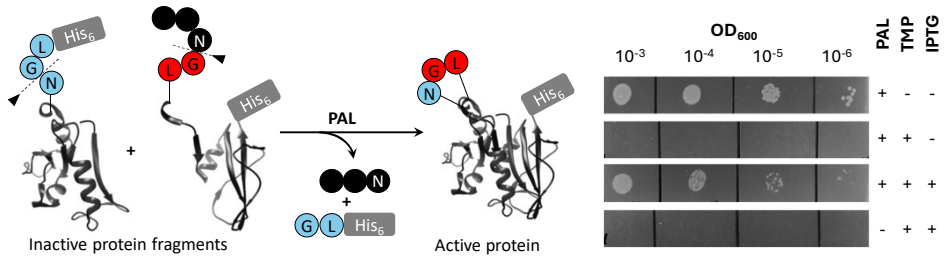
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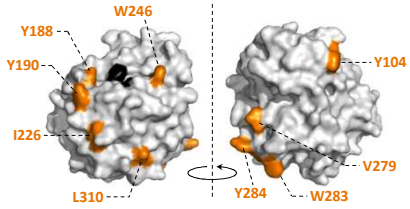
Introduction

- Peptide asaparaginyl ligases (PALs) are ultra-fast transpeptidases that catalyse peptide ligation and head-to-tail cyclisation.[1,2]
- Recombinant OaAEP1b and its engineered derivatives have been increasingly employed for protein and peptide conjugations across various applications.[3,4]
- Protein engineering is central to the development of enzymes into robust biocatalysts, but low mutant screening throughput present a major bottleneck.[5,6]

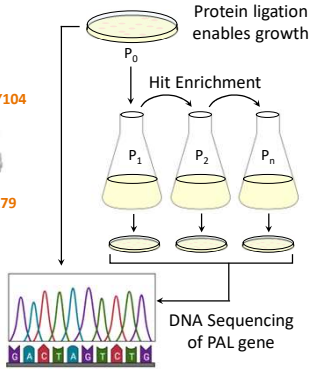
Split-protein reporter links ligase activity to bacterial growth



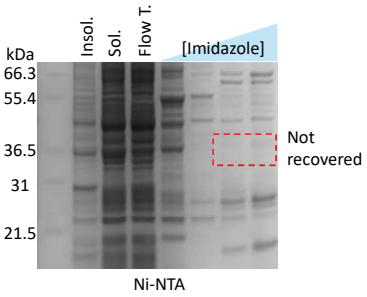
Growth-linked selection identifies s-PAL



Library Size: 589,824 variants

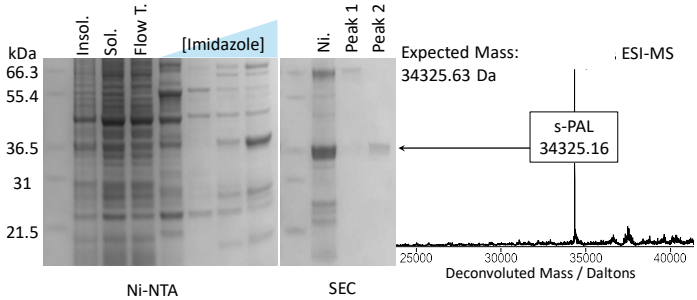


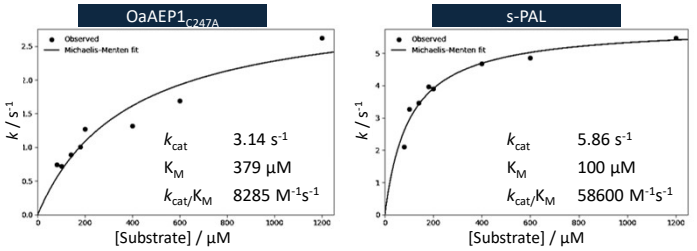
OaAEP1$_{C247A}$



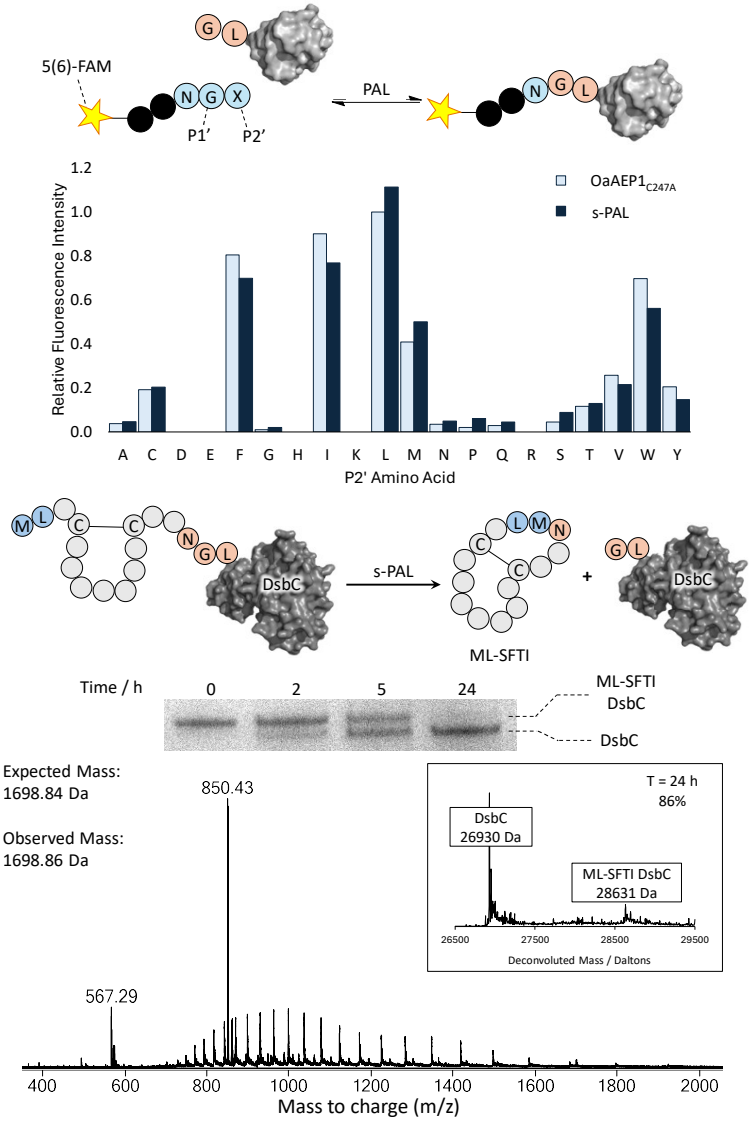
Pos	Amino Acid Options	OaAEP1$_{C247A}$	s-PAL
104	Y H N D	Y	N
188	Y H N D	Y	N
190	Y H N D	Y	N
226	I T N S	I	N
246	W S T R K *	W	W
279	V E K I	V	E
283	W S T R K *	W	K
284	Y H N D	Y	D
310	L Q E V	L	L

s-PAL





Applications to peptide ligation and cyclisation



Conclusion and next steps

- A growth-linked selection platform for rapid PAL engineering was established.
- s-PAL showed improved soluble recombinant protein purification, enhanced kinetics at pH 7.0 and comparable P2' substrate specificity to the parental OaAEP1$_{C247A}$.
- Semi-biosynthetic SFTI-1 analogue demonstrated application.
- Next-generation sequencing will be employed to further elucidate selection dynamics.
- Apply selection system to engineer PAL substrate specificity.

References

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