

Understanding Synthetic Protein Scaffolds

Truncated Scaffolds and Scaffold Domain (PDZ) Protection

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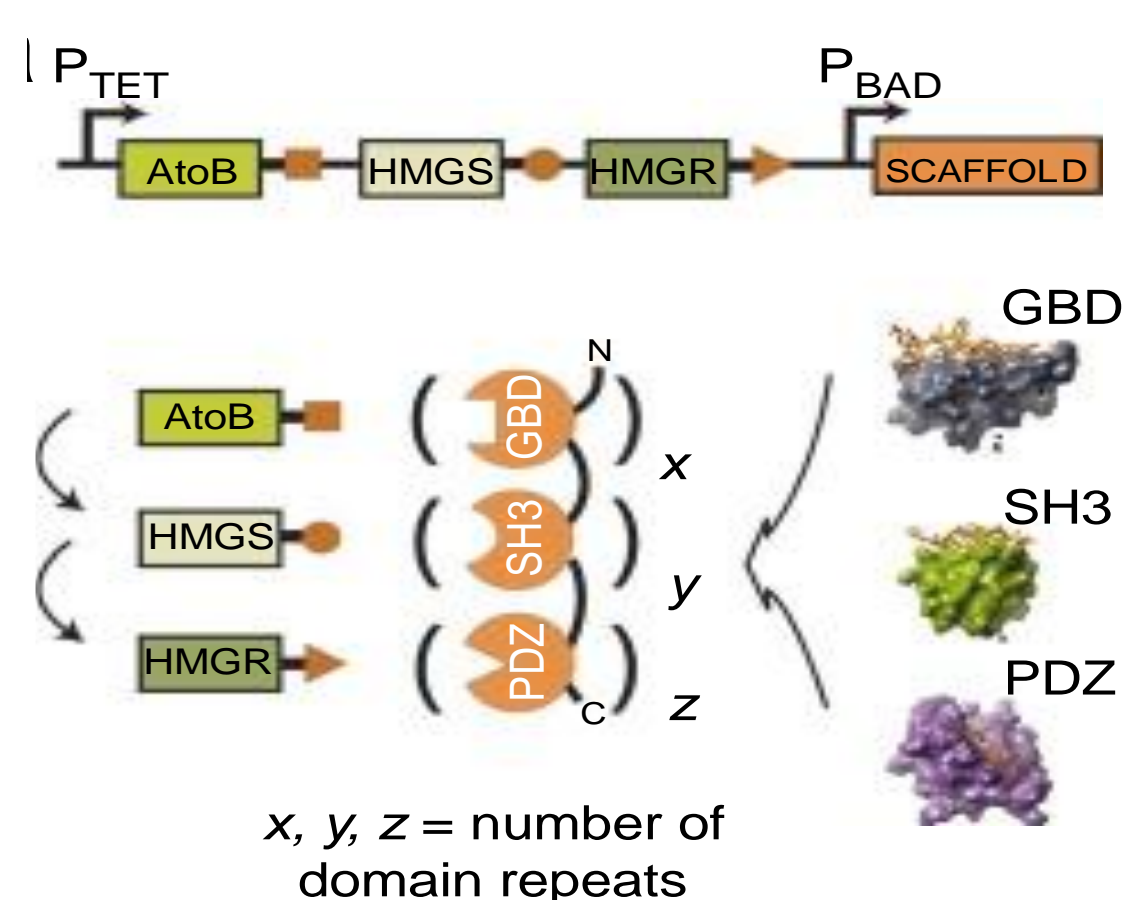
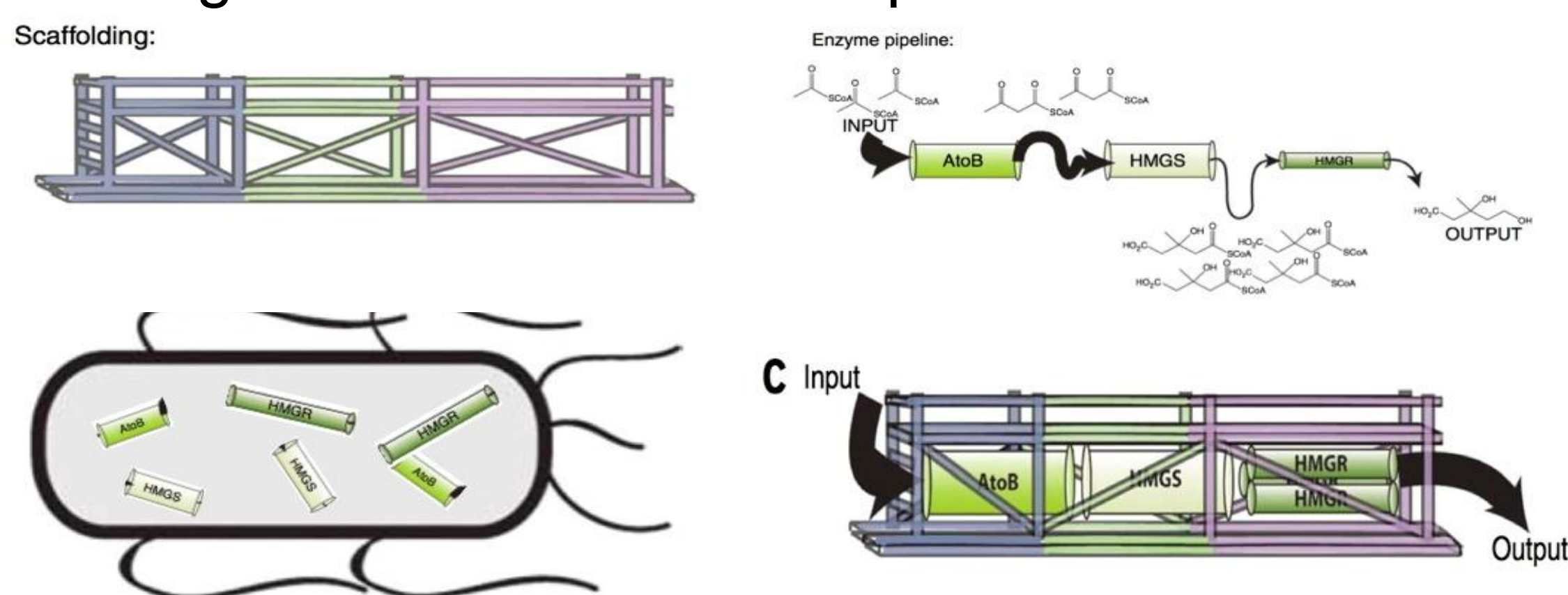
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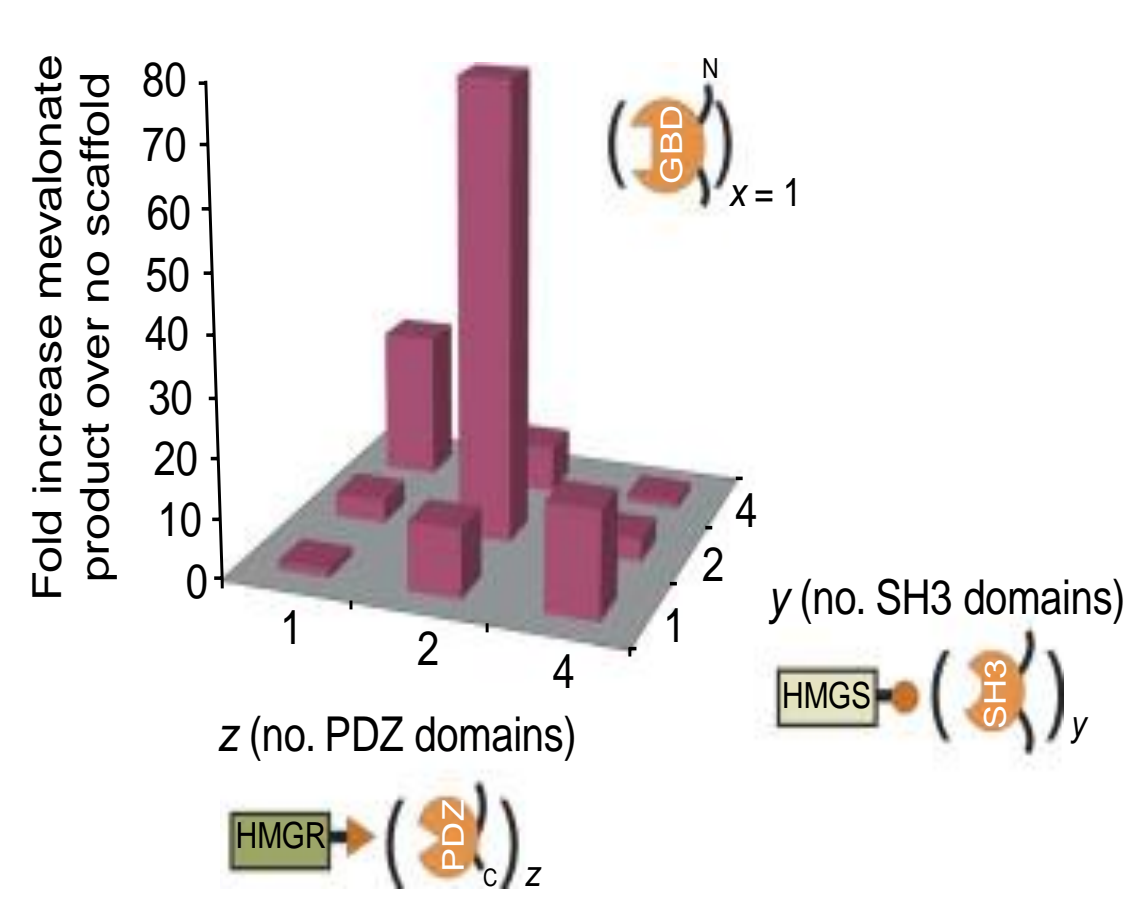
Abstract Synthetic scaffold has shown to be beneficial in increasing the metabolic flux while reducing metabolic load in engineered microbes. In John Dueber's *Nature Biotechnology* paper "Synthetic Protein Scaffolds Provide Modular Control Over Metabolic Flux" ¹, optimize stoichiometry of three mevalonate biosynthetic enzymes recruited to a synthetic scaffolds improved mevalonate production by 77-fold. However, understanding how the scaffold can produce higher titer is extremely complex. For instance, recent discoveries have suggested that internal ribosome binding site (iRBS) is problematic since truncated scaffold is being expressed at higher level than full-length scaffold. Through reconstructing the scaffold DNA without any internal RBS which produces the full-length scaffold and retesting the new scaffold construct would explain how the scaffold function. Additionally, enzyme tagged with PDZ peptide leads to shorter half-life. Enzyme tagged with PDZ peptide can be regulated and thereby reducing activity of off-scaffold enzymes.

Introduction/Background

Synthetic protein scaffolds have been proven to be an effective strategy for enhancing metabolic titers while reducing metabolic load to the production host¹.



The Synthetic scaffolds were constructed with varying number of repeats domains x, y, and z.



Optimal ratio of domains can be found improve production by 77-fold¹.

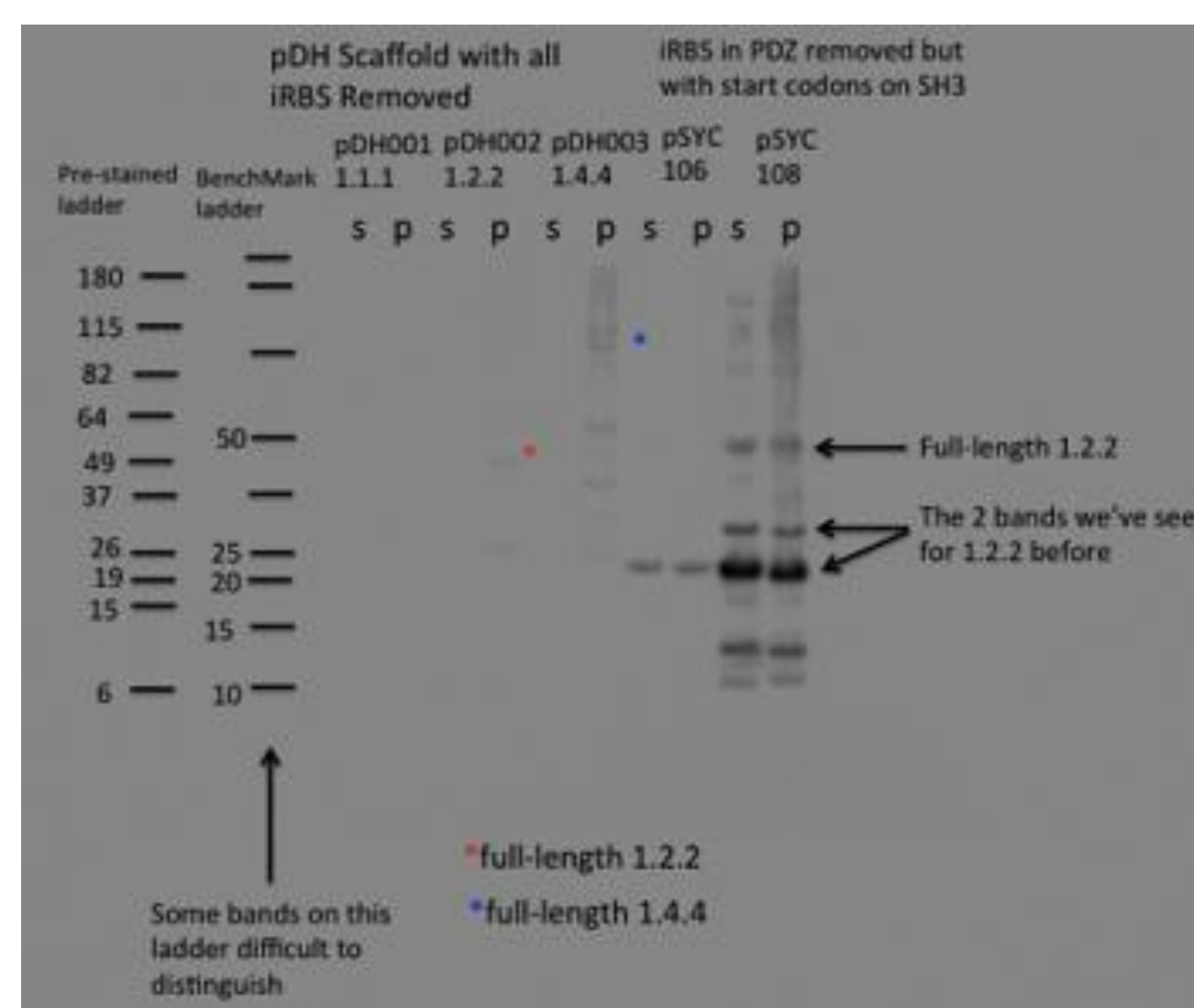
Research

- Internal ribosome binding sites (iRBS) are responsible for truncated scaffolds, which prevent further research until fixed.
- Whether PDZ domain is protecting enzymes tagged with PDZ peptide from degradation.

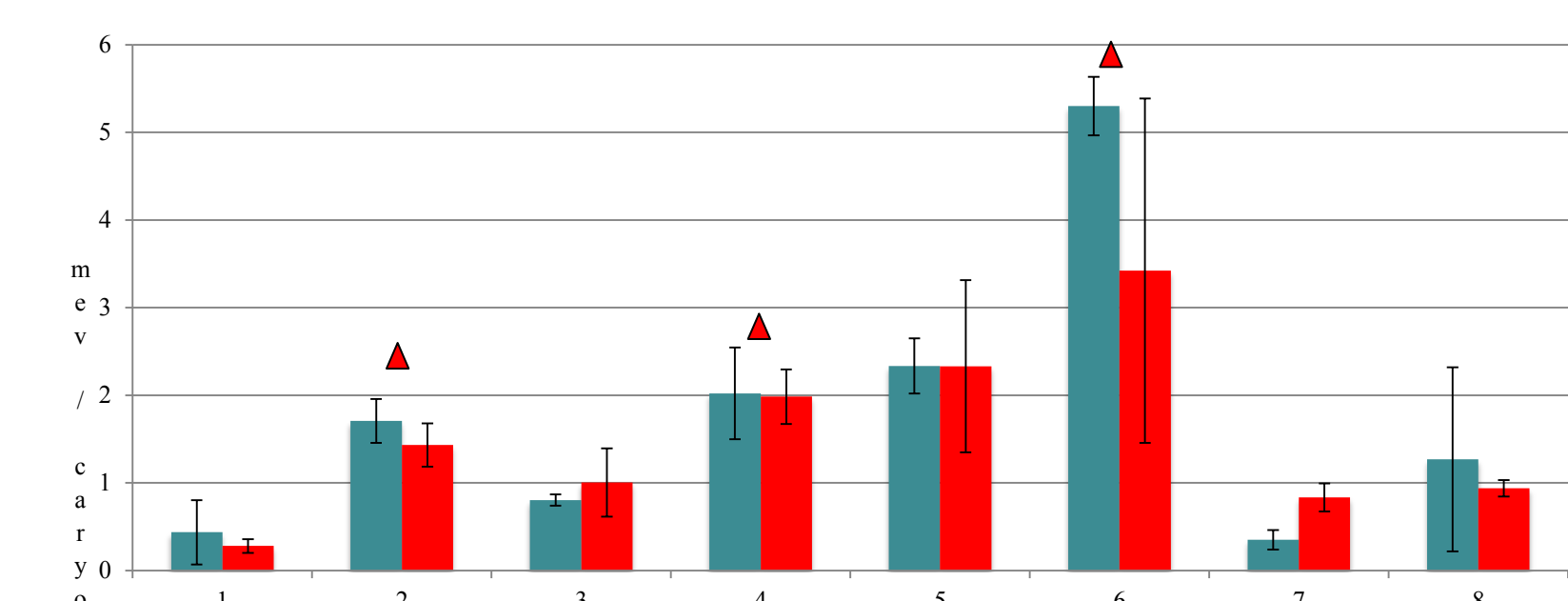
Methods

- Western Bolt for testing full-length scaffolds
- iRBS in the original scaffolds were removed by using RBS calculator.
- 3x Flag Tags were cloned to each plasmid for later processing western blot.
- PDZ Protection
- 1. RFP or RFP-PDZ plasmids were co-transformed with different constructions of the even number plasmids (in **Table 1**).
- 2. Eight samples grew in same condition for mevalonate extraction and GC-MS analysis.

Results/Data Analysis



A Western Blot image shows that we had produced full-length scaffolds but they were barely expressed.



#	Plasmid	Construct
1	pRM178+PDH004 ^a	No scaffold
2	pRM178+PDH005 ^b	No scaffold
3	pGW322+PDH004 ^a	Original Scaffold with iRBS
4	pGW322+PDH005 ^b	Original Scaffold with iRBS removed
5	pGW431+PDH004 ^a	Scaffold with PDZ-iRBS removed
6	pGW431+PDH005 ^b	Scaffold with PDZ-iRBS removed
7	pSY033+PDH004 ^a	HMGR peptide knocked out
8	pSY033+PDH005 ^b	HMGR peptide knocked out

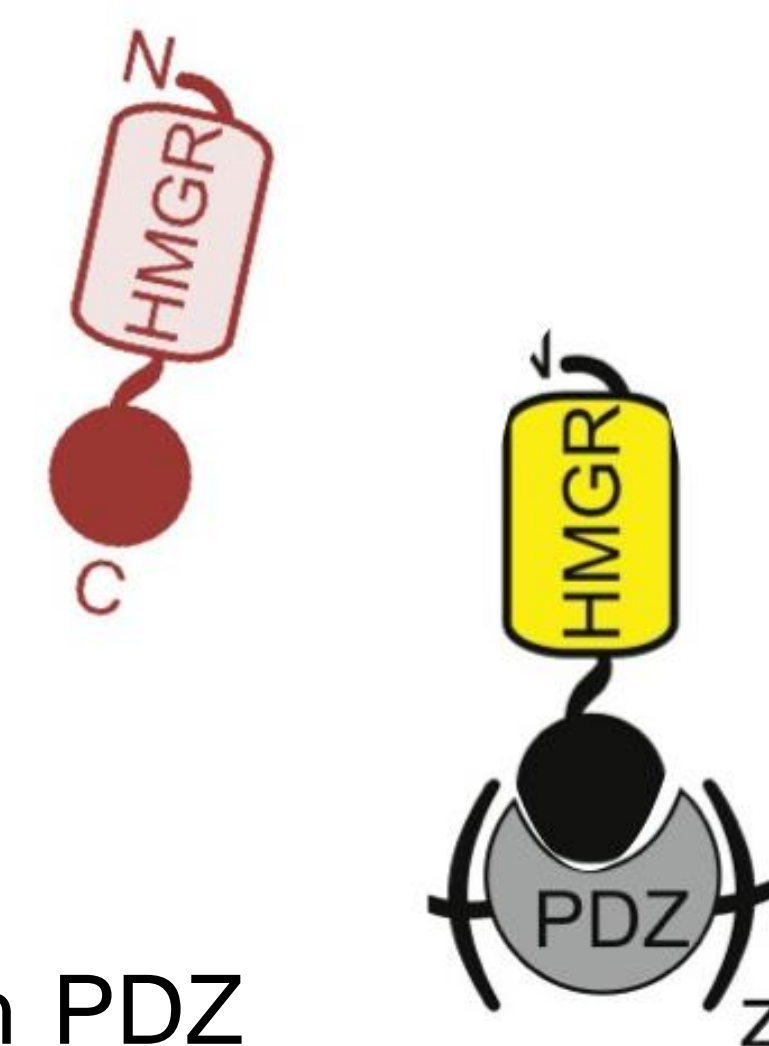
^a PDH004 is the plasmid with RFP.
^b PDH005 is the plasmid with RFP-PDZ.
 Note: All the scaffolds are in the same domains stoichiometry GDB:SH3:PDZ.

Even numbers (2,4,6) of mevalonate titers are higher indicates PDZ domains are protecting HMGR peptide from degradation. (Legend as **Table 1**)

Discussion/Conclusion

-Western Blot

- For further research, due to the refactored scaffolds were not being expressed, we will optimize the scaffold DNA by adding strong RBS. Then, we will test the expression of full-length scaffolds and repeat the mevalonate assay again.



-PDZ domain protection

- PDZ domain is protecting enzymes tagged with PDZ peptide, which helped the previous 77-fold improvement by around 4-fold.
- PDZ domain protection can be useful for regulation. By adding the PDZ peptide to desired enzyme, we can regulate enzymes with PDZ domains.

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References

1. Dueber, J.E., et al., "Synthetic protein scaffolds provide modular control over metabolic flux". *Nat. Biotechnol.* 27, 753–759. (2009)



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