Supplementary Information for

Bioproduced Proteins On Demand (Bio-POD) in hydrogels using *Pichia pastoris*

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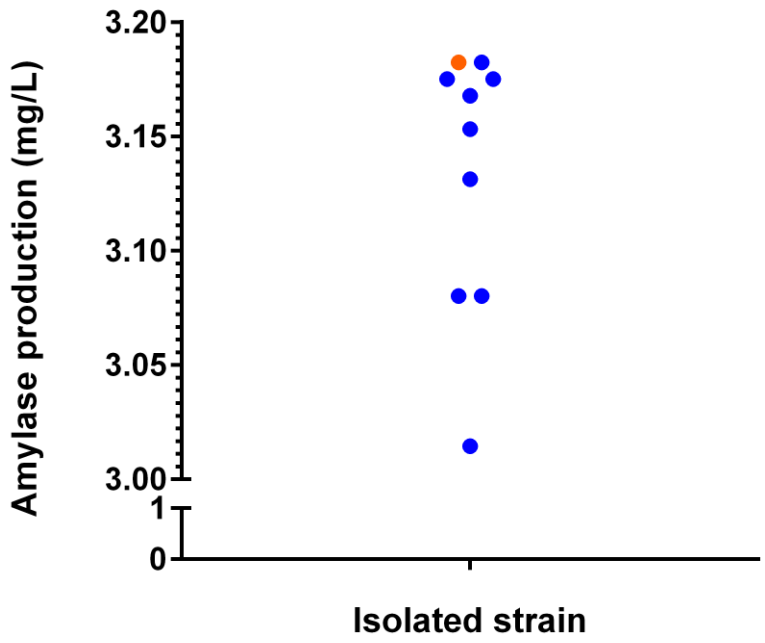
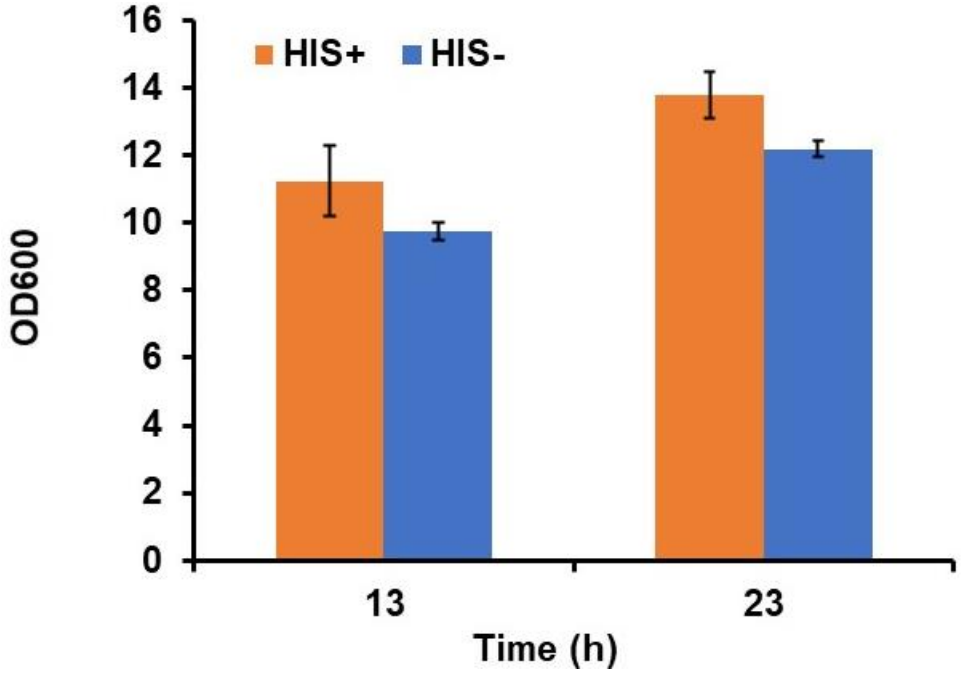
Supplementary Fig. 1



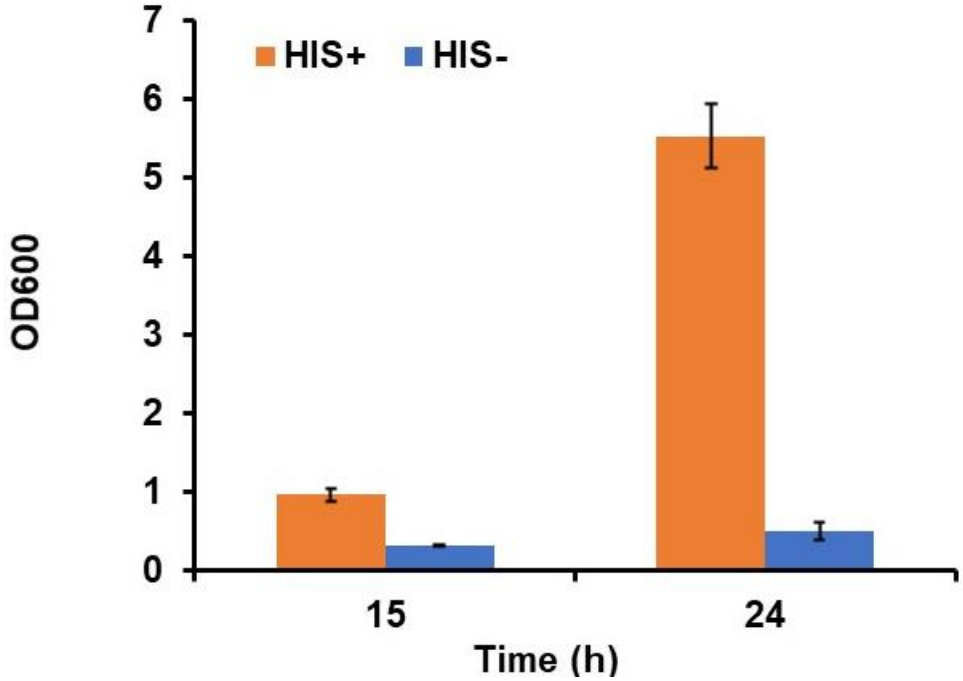
Generation of α-amylase-producing *P. pastoris*. 10 out of 48 zeocin-resistant transformants were cultured in a 96-deep-well microplate and selected based on the cell growth. Secreted α-amylase capacities were evaluated via starch agar plate (measuring the size of the halos) and plate-based starch-iodine assay. Finally, the highest amylase producer (orange dot) was selected.

Supplementary Fig. 2

(A)



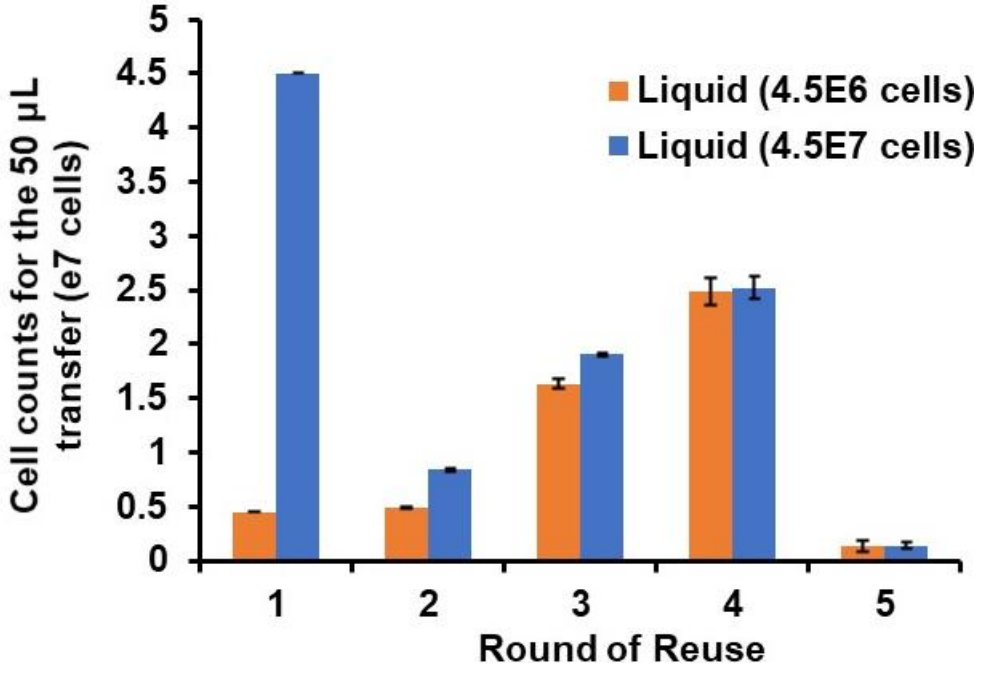
(B)



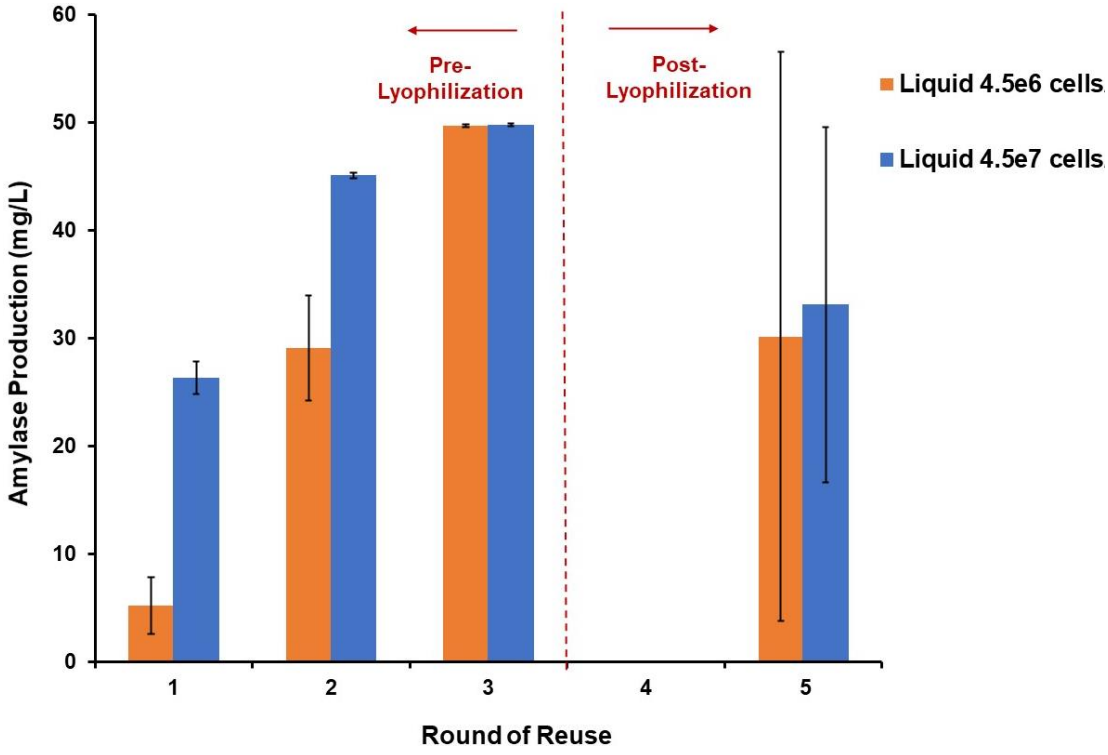
Comparison of growth between Pp01 (HIS^+) and wild-type GS115 (HIS^-). (A) Growth measurement between Pp01 (HIS^+; complementation of GS115 the wild-type *HIS4* gene) and wild-type GS115 (HIS^-) pre-lyophilization. Cells were grown in 3 mL of BMGY medium. (B) Growth measurement between Pp01 (HIS^+) and wild-type GS115 (HIS^-) post-lyophilization. Cells were grown in 3 mL of BMMY methanol medium. All cultures were incubated at 30 °C with an orbital speed of 225 rpm. Data are mean ± s.d.; n = 3 biological replicates.

Supplementary Fig. 3

(A)



(B)



Comparison of yeast cell numbers and amylase production between the conditions using initial inoculum of 4.5 x 10^6 and 4.5 x 10^7 cells for the liquid culture system. (A) Yeast cell counts were measured for the 50 μL subculture used in each round of the amylase liquid culture re-use experiment. (B) Amylase production for each round of reuse. Culture seeded with the initial number of 4.5 x 10^6 and 4.5 x 10^7 cells were separately grown in 3 mL of BMMY media at 30 °C (round 1). For the following round of reuse, 50 μL cultures were taken from the previous batch and transferred to the next batch. The cell numbers in the transfer was calculated by converting OD_{600} value to cell numbers (OD_{600} of 1 for *P. pastoris* is 5 x 10^7 cells/mL). Each of the reuse took 24 h. Data are mean ± s.d.; n = 3 biological replicates.

Supplementary Table 1. List of strains and plasmids used in this study.

Strain/plasmid	Description	Source
***E. coli* strain**		
NEB10β	Δ*(ara-leu) 7697 araD139 fhuA* Δ*lacX74 galK16 galE15 e14- ϕ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL (Str^R) rph spoT1* Δ*(mrr-hsdRMS-mcrBC)*	New England Biolabs
***Pichia pastoris* strain**		
GS115	*his4*	Invitrogen
Pp01	[GS115] *his4::pAOX1-HIS4* (*his4* integration with *HIS4* marker); control strain for SEAP production study	This study
Pp02	[GS115] *his4::pAOX1-tSEAP-tAOX1-HIS4* (*his4* integration with *HIS4* marker); SEAP-producing strain	This study
Pp03	[GS115] *AOX1:pAOX1-tAOX1-ZeoR* (*AOX1* integration with Zeocin resistance gene); control strain for amylase and anti-HER2 production studies	This study
Pp04	[GS115] *AOX1:pAOX1-AmyL-tAOX1-ZeoR* (*AOX1* integration with Zeocin resistance gene); Amylase-producing strain	This study
Pp05	[GS115] *his4:pAOX1-LC-tAOX1-pAOX1-HC-tAOX1-ZeoR-HIS4* (*his4* integration	This study

	with Zeocin resistance gene and *HIS4* marker); anti-HER2-producing strain	
Plasmids		
pPIC9	For construction of plasmid pPIC9-SEAP	Invitrogen
pPICZalphaB-SapL3	For construction of plasmid pPICZα-AmyL	Addgene (Plasmid #78171)
pPICZα	*SapL3* gene, C-myc epitope tag and C-terminal polyhistidine (6xHIS) tag were removed from pPICZalphaB-SapL3	This study
pPICZ-HIS4	For construction of plasmid pPICZ-LC	This study
AbVec-IgG1-hu4D5	For construction of plasmid pPICZ-HC	[1]
AbVec-hIgK-hu4D5	For construction of plasmid pPICZ-LC	[1]
pPIC9-SEAP	For generating a HIS4 integrative cassette employed for SEAP production	This study
pPICZα-AmyL	For generating an integrative cassette containing Zeocin resistance gene employed for amylase production	This study
pPICZ-LC	For construction of plasmid pPICZ_aHER2	This study
pPICZ-HC	For construction of plasmid pPICZ_aHER2	This study
pPICZ-aHER2	For generating an integrative cassette containing full-length of anti-HER2, Zeocin	This study

resistance gene and HIS4 marker employed for anti-HER2 production

Supplementary Table 2. List of primers used in this study.

ID	Description	Sequence (5'->3')
P1	tSEAPpPIC9_F	CTCTCGAGAAAAGAGAGGCTGAAGCT -ATCATCCCAGTTGAGGAGGAGAACC
P2	tSEAPpPIC9_R	GAGGAACAGTCATGTCTAAGGCGAATTA -GTCGGTGGTGCCGGC
P3	pPIC9SEAP_F	TAATTCGCCTTAGACATGACTG
P4	pPIC9SEAP_R	AGCTTCAGCCTCTCTTTTC
P5	pPICalpha_F	GTTTGTAGCCTTAGACATGACTG
P6	pPICalpha_R	AGCTTCAGCCTCTCTTTTCTC
P7	pZ_Gib	ATCTCTCGAGAAAAGAGAGGCTGAAGCT- GTTTGTAGCCTTAGACATGACTGTTCCT
P8	AmyL_F	GGTATCTCTCGAGAAAAGAGAGGCTGAAGCT- GCTAATTTGAATGGTACTTTGATG
P9	AmyL_R	GAGGAACAGTCATGTCTAAGGCTACAAAC- TTATCTTTGAACATAAATAGAAACAGAAC
P10	HIS_F	GGGACGCTCGAAGGCTTTAATTTGCAAG-CGCTCTCCCTTATGCGACTC
P11	HIS_R	CTGGCCTTTTGCTCACATGTTGGTCTCCAG-CGTTCGTTTGTGCAAGCTTATC

P12	pZHIS_F	CTGGAGACCAACATGTGAGCAAAAGG
P13	pZHIS_R	CTTGCAAATTAAAGCCTTCGAGCGTC
P14	Light_F	GTATCTCTCGAGAAAAGAGAGGCTGAAGCT-GACATCCAGATGACCCAGTC
P15	Light_R	GAGGAACAGTCATGTCTAAGGCTACAAAC-ACACTCTCCCCTGTTGAAG
P16	PICZlight_F	GTTTGTAGCCTTAGACATGACTG
P17	PICZlight_R	AGCTTCAGCCTCTCTTTTCTC
P18	Heavy_F	GTATCTCTCGAGAAAAGAGAGGCTGAAGCT-GAAGTGCAGCTGGTGGAATC
P19	Heavy_R	GAGGAACAGTCATGTCTAAGGCTACAAAC-TCATTTACCCGGGGACAGG
P20	scrHeavy_R	GAAGCTATGGTGTGTGGGG
P21	LightHeavy_F	CCTTCGTTTGTGCGGATCC
P22	EcoRIHeavy_F	GGAATTC-GATCTAACATCCAAAGACGAAAGGTTG
P23	EcoLightHeavy_R	GGAAT-TCTCACTTAATCTTCTGTACTCTGAAGAG

The underlined sequence indicates that the nucleotides used to be annealed to the template for PCR amplification.

Supplementary Table 3. gBlock of codon-optimized amylase used in this study.

Description	Sequence (5'->3')
CO_amyL	GCTAATTTGAATGGTACTTTGATGCAGTATTTCGAGTGGTACATGC
	CTAACGACGGACAGCACTGGAAGAGATTGCAGAACGACTCCGCC
	TACTTGGCTGAGCACGGAATTACTGCTGTCTGGATCCCTCCAGCT
	TACAAGGGAACTTCTCAGGCTGACGTTGGTTACGGTGCTTACGAC
	TTGTACGACCTTGGTGAGTTCCACCAAAAAGGTACTGTCCGTACC
	AAATATGGTACCAAGGGTGAGTTGCAGTCCGCCATTAAGTCCTTG
	CACTCCAGAGACATCAACGTCTACGGTGACGTTGTCATCAACCAC
	AAGGGTGGTGCCGATGCCACTGAAGATGTTACTGCTGTCGAGGT
	CGACCCAGCTGATAGAAACCGTGTCATCTCCGGAGAGCACAGAA
	TCAAGGCTTGGACCCATTTCCATTTCCCAGGTCGTGGTTCCACCT
	ACTCCGACTTCAAATGGCACTGGTACCACTTCGATGGTACCGACT
	GGGACGAGTCCAGAAAATTGAACCGTATTTACAAGTTCCAAGGT
	AAAGCCTGGGACTGGGAGGTTTCCAATGAGAACGGTAATTATGAT
	TACTTGATGTACGCTGACATTGACTACGATCACCCAGATGTCGCT
	GCTGAGATCAAGAGATGGGGTACCTGGTACGCCAACGAGCTTCA
	GTTGGACGGTTTCCGTTTGGACGCCGTCAAGCACATCAAATTTTC
	TTTCTTGAGAGACTGGGTCAACCACGTCAGAGAAAAGACCGGTA

AGGAGATGTTCACCGTCGCCGAGTACTGGCAGAACGATCTTGGT

GCTTTGGAAAACTATTTGAACAAGACTAACTTTAACCATTCTGTT

TTCGACGTTCCACTTCACTACCAGTTTCATGCCGCCTCTACCCAG

GGTGGTGGTTACGACATGAGAAAGTTGTTGAACTCCACCGTTGT

CTCCAAGCACCCTCTTAAGGCCGTTACCTTTGTCGACAATCACGA

CACCCAGCCTGGTCAATCCTTGGAGTCCACTGTTCAGACTTGGTT

CAAGCCATTGGCTTACGCCTTTATTTTGACTAGAGAGTCCGGATA

CCCACAGGTTTTCTACGGTGACATGTACGGTACCAAAGGAGACTC

CCAAAGAGAGATTCCTGCTTTGAAGCATAAGATCGAACCTATTTT

GAAGGCTCGTAAACAGTACGCCTACGGAGCTCAGCACGACTACT

TCGATCACCACGATATCGTCGGTTGGACTAGAGAGGGAGACTCTT

CTGTCGCCAACTCTGGTTTGGCCGCTTTGATTACTGATGGTCCAG

GAGGTGCCAAGAGAATGTACGTCGGACGTCAGAACGCTGGTGAG

ACCTGGCACGACATTACCGGTAACAGATCCGAGCCAGTCGTTATC

AACTCCGAGGGATGGGGTGAGTTCCATGTTAACGGTGGTTCTGTT

TCTATTTATGTTCAAAGATAA

Reference

[1] A. W. Nguyen, K. C. Le, J. A. Maynard, Identification of high affinity HER2 binding antibodies using CHO Fab surface display, Protein Engineering, Design and Selection. 31 (2018) 91-101. http://dx.doi.org/10.1093/protein/gzy004.