
Supplementary information

Yeast-based bioproduction of disulfide-rich peptides and their cyclization via asparaginyl endopeptidases

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Yeast-based bioproduction of cyclic disulfide-rich peptides and their cyclization via asparaginyl endopeptidases

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a



pPICZ-alpha-SFTI-KLK5

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNGLLFINTT
IASIAAKEEGVSLEKRGFCHRSYPPECWPNGLPHHHHHHHH

pPICZ-alpha-[G22N] cVc1.1

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNGLLFINTT
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pPICZ-alpha-MCoTI-II

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNGLLFINTT
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b



pET-15-ubiquitin-[C247A]OaAEP1_b

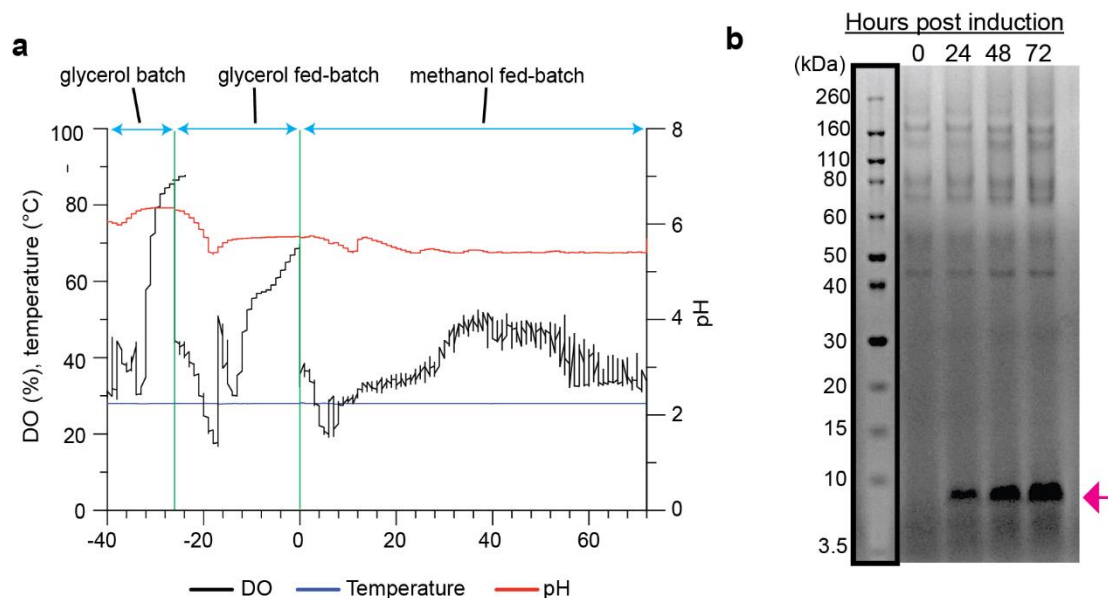
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VNQRDADLLHLWEKFRKAPEGSARKEVAQTQIFKAMSHRVHIDSSIKLIGKLLFGIEKCTEILNAVRPA
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pET-15-ubiquitin-MCoAEP2

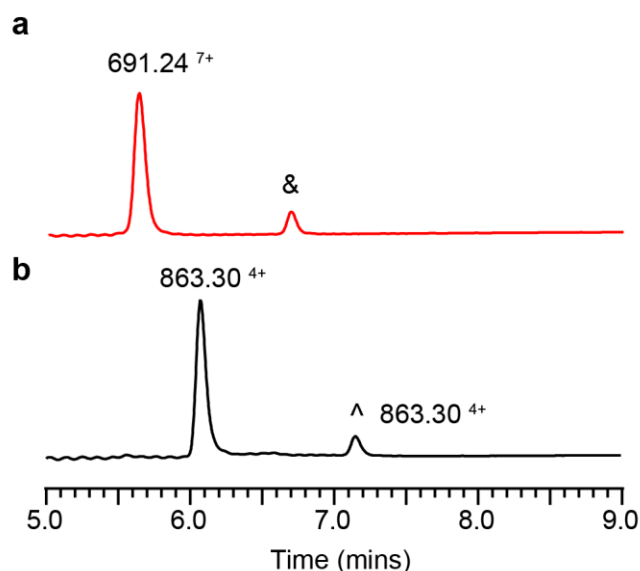
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DSDRHNLTESLRQQYELVKRRTLNDYTVYGSHVMQYGDITLNKNALFSYLGTDPANENNTFVESNSLR
PTTKVTNQRDADMVHFWEKFRKAPEGSAQKIEAQKHFEAMSHRVHIDNSVKLIGKLLFGIEKGPEVLN
AVRPTGQPLVNNWDCLKNMVRSEFETHCGSLSQYGMKHMRSFANLCNAGIRNEQMAEASAQACVSVSPGP
WSSLHKGFTA

Supplementary Fig. 1 Overview of construct design for CPs and recombinant AEPs. a

The amino acid sequences of *S. cerevisiae* α-mating factor secretion signal (grey colored letters) fused on the N-terminus of cyclic peptide precursors (red colored letters) followed by a C-terminus His tag (blue colored letters). **b** The amino acid sequences of AEP zymogens represented (green colored letters) fused to the C-terminus of ubiquitin solubility protein (grey colored letters) and a His tag was placed on the N-terminus of the ubiquitin protein (blue colored letters).



Supplementary Fig. 2 Bioreactor production of MCoTI-II CPs. **a** Parameters of a bioreactor bioproduction of MCoTI-II precursors showing temperature, dissolved oxygen (DO) and pH for the growth phases of glycerol batch, glycerol-fed batch and methanol fed batch. **b** SDS-PAGE analysis of MCoTI-II CP accumulation at different time point post methanol induction. Expected bands indicated by a magenta colored arrow.



Supplementary Fig. 3 HPLC-UV-HRMS chromatograms. **a** IMAC purified fractions containing MCoTI-II precursor before AEP-mediated cyclization. **b** RP-HPLC purified recombinantly produced MCoTI-II. & unidentified *P. pastoris* contaminant and ^ β - aspartyl isomers¹.

- 1 Hernandez, J. F. *et al.* Squash trypsin inhibitors from *Momordica cochinchinensis* exhibit an atypical macrocyclic structure. *Biochemistry* **39**, 5722-5730 (2000).