SUPPORTING INFORMATION

Parallel Sampling of Nanoliter Droplet Arrays for Noninvasive Protein Analysis in Discrete Yeast Cultivations by MALDI-MS

Dominik Haidas[1], Marta Napiorkowska[1], Steven Schmitt[1], and Petra S. Dittrich[1,*]

[1]Bioanalytics Group, Department of Biosystems Science and Engineering, ETH Zürich, Mattenstr. 26, 4058 Basel, Switzerland, and [2]Bioprocess Laboratory, Department of Biosystems Science and Engineering, ETH Zürich, Mattenstr. 26, 4058 Basel, Switzerland

Brazzein production and purification. A 5 ml YPD preculture of brazzein-secreting *K. phaffii* was cultivated until stationary phase and used to inoculate 500 ml YPD medium at a dilution of 1:1000. Upon reaching stationary phase, the production culture was centrifuged (3,500 g, 20 min), the supernatant was harvested, adjusted to pH of 7.0, and filtered through a bottle-top filter (0.22 µm pore size, polyether sulfone membrane, Stericup, Merck Millipore). The purification was done using an ÄKTA explorer chromatography system (GE Healthcare) operated with Unicorn software version 5.31. The supernatant was loaded onto an XK 16/20 column packed with 10 ml of Ni Sepharose Excel (GE Healthcare) and equilibrated with 5 column volumes (CV) of wash buffer (500 mM NaCl, 20 mM sodium phosphate, pH 7.4). The column was washed with 10 CV of wash buffer before eluting the peptide with 3 CV of elution buffer (500 mM NaCl, 500 mM imidazole, 20 mM sodium phosphate, pH 7.4). The eluent was loaded onto an XK 50/30 column packed with 300 ml of Sephadex G-25 (GE Healthcare), which was equilibrated with 5 CV of desalting buffer (20 mM ammonium carbonate, pH 7.4), and eluted with 2 CV of desalting buffer. The eluted fractions corresponding to brazzein (monitored at 280 nm absorbance) were collected, pooled, frozen at 80°C for 2 h, and lyophilized using a manual vacuum freeze-dryer (Alpha 1-2 LD plus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) connected to a vacuum pump (RC6, Vacuubrand, Wertheim, Germany). The freeze-dried peptide powder was dissolved in 1 ml of phosphate buffer (10 mM sodium phosphate, 1.8 mM potassium phosphate, 137 mM NaCl, and 2.7 mM KCl, pH 7.4) and subjected to preparative high-performance liquid chromatography (HPLC) using a Prontosil PREP 2025 C18 column (10 mm, 250 mm × 20 mm) with a guard column (Bischoff Chromatography, Leonberg, Germany). The column temperature was maintained at 30 °C. The mobile phase was composed of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B). The flow rate was maintained at 10 ml min^{-1}. The column was equilibrated on 0% B for 6 min followed by the linear gradient from 0% B to 56% B for 40 min. The fractions corresponding to brazzein (monitored at 210 nm absorbance) were pooled, frozen at -80°C for 2 h, and lyophilized as before. The freeze-dried peptide powder was dissolved in 1 ml of water and stored at -20°C. For quantification of brazzein, the brazzein sample was subjected to a reversed-phase HPLC (Agilent Technologies) using a ReproSil-Pur C18-AQ column (3 mm, 150 mm × 2.1 mm; Dr. Maisch GmbH). The mobile phase was composed of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B) and was run on 0% B for 3 min followed by a linear gradient from 0% B to 50% B for 25 min at a flow rate of 0.3 ml min^{-1}. The sample injection volume was 50 µl and the column temperature was maintained at 40 °C. The concentration was measured using the area under the curve at 205 nm and brazzein-specific molar extinction coefficient (258506 M^{-1} cm^{-1}).[1,2]

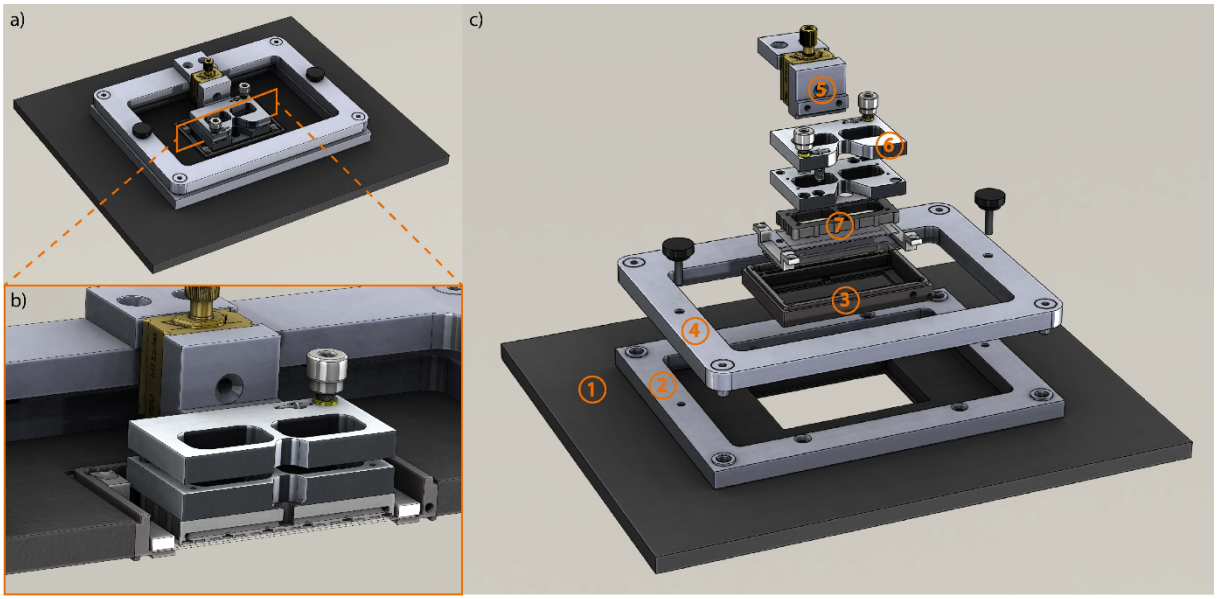

Figure S1. System for aligning the top and bottom plate. a) Overview of the whole system. b) Cut and zoom in the top and bottom slide holder. c) Individual components of the plate alignment system mounted on the microscopy stage (1). The bottom part of the system consists of a frame (2) that is fixed to the microscopy stage. At the four edges of this frame, the bottom part of locating block sets (TPCAT12C, Misumi) are inserted. The bottom slide holder (3) can be placed directly into the center of the microscopy stage, and serves additionally as oil bath. The upper part of the alignment systems consists of the upper frame (4), which is fixed to a manual precision linear stage (5) (MT X, Newport) to allow for vertical movement. To also correct for horizontal tilts, we integrated a tilting stage (6), custom-made from a kinematic mirror mount (KMS, Thorlabs). At the edges of the upper frame the top parts of the locating block sets with tapered pins (TPCAT12C, Misumi) are placed. The top plate of 60x25 mm^2 size is hold in the upper frame using vacuum. Therefore, 3D-printed hollow ring (7) (custom design, printed by Protolabs, material: Accura ABS black) is used and connected to vacuum supply tubing and the vacuum pump (here not shown).

Table S1. Recipe of the HCD medium. For further information, see ref. (3). The individual solutions were premixed and stored at 4 °C. Before the experiment the HCD medium was mixed together as specified by the volume ratio.

	Volume ratio (%)	**Mass concentration (g/L)**	**Molar concentration (mM)**
Glucose solution	**22.5**		
Glucose		200	1110.1
Glutamate solution	**8.1**		
Monosodium glutamate		50	295.6
Amino acid solution	**9.9**		
Glutamine		5	34.0
Histidine		1.75	11.3
Leucine		5.5	41.9
Lysine		6	41.0
Methionine		2	13.4
Phenylalanine		2.5	15.1
Serine		1.875	17.8
Threonine		1	8.4
Uracil		2	17.8
YNB solution	**27.0**		
YNB		15	108.6
Inositol solution	**2.1**		
Inositol		10	55.5
Buffer solution	**2.0**		
MES		195.2	1000.0
Ammonium sulfate		1	7.6
Sodium dihydrogen phosphate		1	8.3
Water	**28.4**		

Table S2 List of chemicals

Chemical	**Supplier**
Acetonitrile	Sigma-Aldrich
Ammonium bicarbonate	Sigma-Aldrich
Ammonium sulfate solution (3.2 M)	LabForce, Muttenz, Switzerland
Dextrose	Sigma Aldrich
D-Glucose	Sigma-Aldrich
Di-potassium hydrogen phosphate	Carl Roth
Di-sodium hydrogen phosphate	Carl Roth
Glutamic acid	Sigma Aldrich
HFE 7500	3M, Maplewood, USA
Histidine	Sigma Aldrich
Imidazole	Applichem GmbH, Darmstadt, Germany
Inositol	Sigma Aldrich
Leucine	Sigma Aldrich
Lysine	Fluka (Honeywell),Charlotte, USA
MES	TCI Deutschland GmbH
Methionine	Fluka (Honeywell),Charlotte, USA
Monosodium glutamate	Sigma Aldrich
Peptone	Becton Dickinson
Phenylalanine	Sigma Aldrich
Potassium chloride (KCl)	Applichem GmbH, Darmstadt, Germany
Potassium di-hydrogen phosphate	Applichem GmbH, Darmstadt, Germany
Serine	Sigma Aldrich
Sodium chloride (NaCl)	Applichem GmbH, Darmstadt, Germany
Sodium dihydrogen phosphate dihydrate	Carl Roth, Merck KGaA
Threonine	Sigma Aldrich
Trifluoroacetic acid (TFA)	Sigma-Aldrich, Thermo Fisher
Uracil	Sigma Aldrich
Yeast extract	Becton Dickinson
Yeast nitrogen base (YNB)	Becton Dickinson
Yeast extract peptone dextrose (YPD) broth	Sigma Aldrich
Zeocin	Thermo Fisher
α-cyano-4-hydroxy-cinnamic acid (CHCA)	Sigma-Aldrich

References:

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(3) Roberts, T. M.; Kaltenbach, H.-M.; Rudolf, F. Development and Optimisation of a Defined High Cell Density Yeast Medium, BioRvix, doi: 10.1101/846006.