Electronic Supplementary Information

Materials

Yeast extract (Cat. No.: 210929), peptone (Cat. No.: 211677), and yeast nitrogen base (Cat. No.: 291920) were purchased from Becton, Dickinson, and Company. K_2HPO_4 (Cat. No.: P3786), KH_2PO_4 (Cat. No.: P5655), and glycerol (Cat. No.: G5516) were purchased from Sigma-Aldrich. D-biotin (Cat. No.: 131595) was purchased from Life Technologies. Methanol (Cat. No.: MX0488-1) was purchased from EMD Millipore. Conjugation of horseradish peroxidase (HRP) to ELISA detection antibody was performed using the HRP-Antibody All-In-One Conjugation Kit (Solulink; Cat. No.: A-9002-001). QuantaBlu™ Fluorogenic Peroxidase Substrate Kits (Cat. No.: 15169) and Microfluor® 2Black Flat Bottom Microtiter® Plates (Cat. No.: 7805) were purchased from Thermo Scientific. Amicon® Ultra-15 Ultracel®-3k centrifugal filters (Cat. No.: UFC900396) were purchased from Merck Millipore Ltd. HT Protein Express Reagent Kits (Cat. No.: CLS960008) and HT Protein Express LabChips (Cat. No.: 760449) were both purchased from PerkinElmer. Perfusostat chips (Cat. No.: CCPST-1), microbioreactor control modules (Cat. No.: MCM-001), "Quad Control Hubs" (Cat. No.: MBS-004), and associated peripherals were purchased from Pharyx Inc.

Perfusion culture microscale bioreactor

Microbioreactors with a perfusion filter were used for these experiments. A detailed description of the fabrication process, device structures, and device operation are provided in previous reports.[13,32] Salient differences between the current and previous devices are highlighted here. The fluid input/output ports were consolidated on one edge of the device for easy interfacing with the microbioreactor control module. Filter integration was achieved by bonding the perimeter of a 1 cm diameter polyethersulfone filter (0.22 μm; Pall Supor-200) to the bottom of a growth chamber section and over an outlet channel connected to an output port of the device.

Online optical density was estimated from the transmission of 630 nm LED light through the growth chamber.[12,13] The internally referenced optical density measurement structure comprised two opposing cylindrical pedestals, one on the bottom of the growth chamber and one in the corresponding mixing chamber headspace, with the mixer membrane in between. The two pedestals served to reduce the optical path length through the culture to 508 μm to provide an effective working range of 0 – 1.2 absorbance units corresponding to a yeast cell density range of 0 – 6.5 x 10^8 cells/mL. Optical density was determined using two measurements, one with the mixing membrane contacting the upper pedestal (to measure the transmission through the culture), and another with the mixing membrane contacting the lower pedestal (for a reference measurement without the culture).

The microfluidic bioreactor was operated by inserting it into a microfluidic control module with a rack of polypropylene bottles containing media for the experiment. The microfluidic control module supplied pneumatic signals to actuate the PDMS microvalves and membrane mixer and also provided all of the optics and electronics for sensor readout and bioreactor control. Temperature was controlled using resistive heaters contacting the top and bottom of the microbioreactor to uniformly heat the device and prevent condensation, and a cooling fan to allow control within 0.1 °C for setpoints 3 °C above ambient temperature. Each microbioreactor control module operated a microfluidic bioreactor independently and in a fully automated fashion with pre-programmed fluid addition/removal and integrated control loops. Four microbioreactor control modules were supplied with regulated pressure and electrical power by a control hub that also provided communication with a host computer for experimental setup and data visualization.

The flow of fluids into the growth chamber was controlled using peristaltic pump valves, sized to allow single injections of 800 nL (on average) with a maximum flow rate of 2.3 mL/h set by the volume and response time of the valves. Perfusion output from the growth chamber was accomplished using a scheme similar to that used for evaporation compensation as described in [13]. Briefly, fluid was injected

into the growth chamber at the desired flow rate, causing the growth chamber to accumulate in volume. After a set accumulation threshold of approximately 5 µL, the excess fluid was removed through the perfusion filter by pressurizing a growth chamber section and opening the perfusion output valve until the growth chamber section was fully depressed, normalizing the volume of the growth chamber back to 1 mL.

Sensor data from each microbioreactor were acquired, plotted, and exported using the MBS Dashboard software (Pharyx, Inc.; version 1.0.9.0ac). Post-processing of these data was performed using MATLAB (The MathWorks, Inc.; R2014a).

Measurement of protein expression/titer

Titers of secreted recombinant protein in perfused cultivation fluid were quantified by both capillary electrophoresis (hGH and IFNα-2b; PerkinElmer Caliper LabChip® GXII) and sandwich ELISA immunoassay (hGH). All samples were pre-concentrated using Amicon® Ultra-15 Ultracel®-3k centrifugal filters.

An ELISA for detection of hGH and determination of titers in cultivation supernatants was validated for use on a Tecan Freedom EVO® 150 liquid handling system equipped with an MCA96 head. The ELISA was performed using Microfluor® 2Black Flat Bottom Microtiter® plates coated by liquid-handler with mouse anti-human hGH IgG1 (MA1-21649, ThermoFisher Scientific) as capture antibody (10 µg/mL). Following the addition of either cultivation supernatants or protein standards, a different mouse anti-human hGH IgG1 (MA1-21650, ThermoFisher Scientific) conjugated to horseradish peroxidase (HRP) was used for detection (250 ng/mL). Signal was developed using the QuantaBlu™ Fluorogenic Peroxidase Substrate Kit. Plates were analyzed using a Tecan Infinite® M200PRO at excitation and emission wavelength settings of 325 nm and 420 nm, respectively.

Media preparation

Buffered glycerol-complex medium (BMGY) comprised 10 g/L [1% (w/v)] yeast extract, 20 g/L [2% (w/v)] peptone, 13.4 g/L [1.34% (w/v)] yeast nitrogen base, 2.299 g/L [13.2 mM] K_2HPO_4, 11.814 g/L [86.81 mM] KH_2PO_4, 0.4 mg/L [4 x 10^{-3}% (w/v)] D-biotin, and 40 mL/L [4% (v/v)] glycerol. The yeast extract, peptone, yeast nitrogen base, K_2HPO_4, KH_2PO_4, and D-biotin concentrations in BMMY were identical to those in BMGY. The methanol concentration in BMMY was 10 mL/L [1% (v/v)]. For both BMGY and BMMY, all components were dissolved in 18.2 MΩ•cm water (Barnstead™ NANOpure Diamond™, Thermo Scientific), mixed, and sterilized as reported previously.[25]

Working cell bank (WCB) creation

Pichia pastoris strains secreting hGH and IFNα-2b under the control of the AOX1 promoter were streaked from frozen clonal master cell banks (MCBs) onto solid Yeast Extract Peptone Dextrose (YPD) media.[25] Colonies were allowed to develop for ~3 days at room temperature. 1 µL of cells from a given plate was used to inoculate 10 mL of liquid BMGY and then grown to an offline cell density of ~3 OD (600 nm; 1 cm pathlength) at 25 °C with shaking at 250 rpm. A sufficient volume of this culture was then harvested by centrifugation (23 °C, 1500 x g, 3 min) to permit inoculation of 200 mL of fresh liquid BMGY at an offline cell density of 0.1 OD/mL. The second liquid culture was grown to an offline cell density of ~5 OD/mL at 25 °C with shaking at 250 rpm. Following centrifugation (23 °C, 1500 x g, 7 min) to harvest the entire 200 mL culture, fresh liquid BMGY was mixed with the pellet in sufficient volume to yield a cell suspension at twice the intended WCB density. 1:1 dilution of this suspension with sterile 40% (v/v) glycerol yielded WCB aliquots of the desired cell density. All WCB aliquots were stored at -80 °C until use.

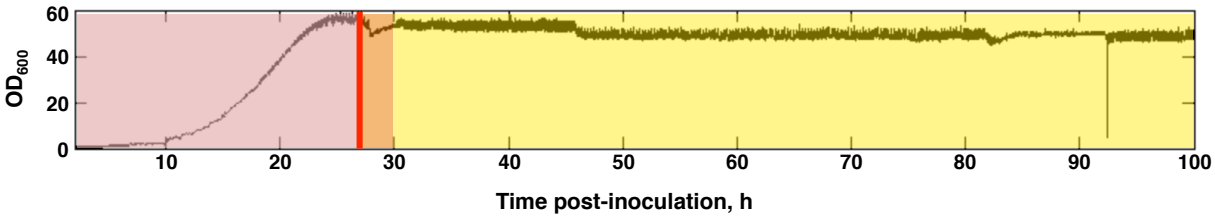

Fig. S1: Schematic of a typical perfusostat cultivation time-course. Inoculation from frozen aliquots of a working cell bank occurred at time 0 h. Red region corresponds to the outgrowth phase for biomass accumulation using glycerol as the primary carbon source prior to induction. Red line denotes the time of induction, when glycerol was substituted for methanol as the primary carbon source by media exchange. Orange region corresponds to the transition phase during which biomass adapted to utilizing methanol. Yellow region corresponds to the protein expression phase. The entire cultivation time-course was operated under perfusion conditions.

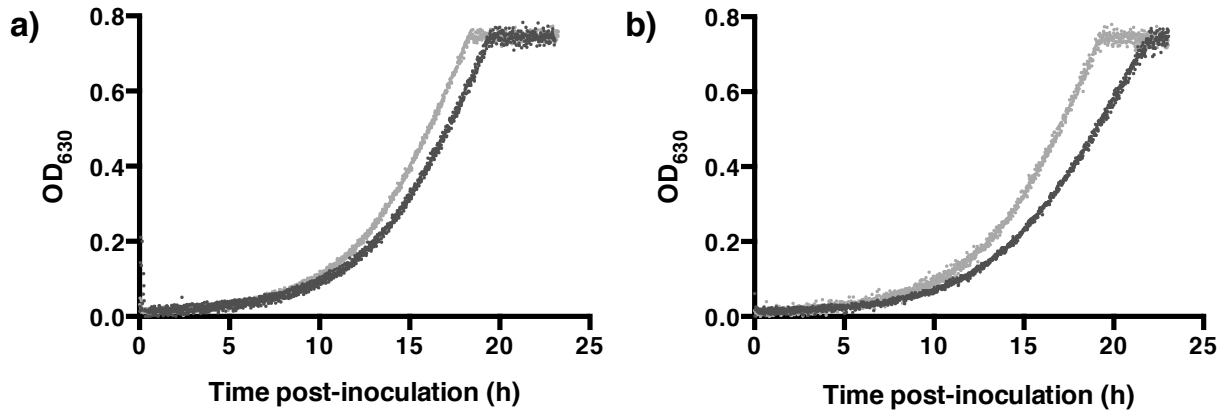

Fig. S2: Replicate growth profiles generated by parallel perfusostat cultivations of both **a)** hGH-secreting and **b)** IFNα-2b-secreting strains of *P. pastoris*. Cultures were inoculated at an offline OD_{600} of ~0.1 and grown on non-inductive BMGY media.

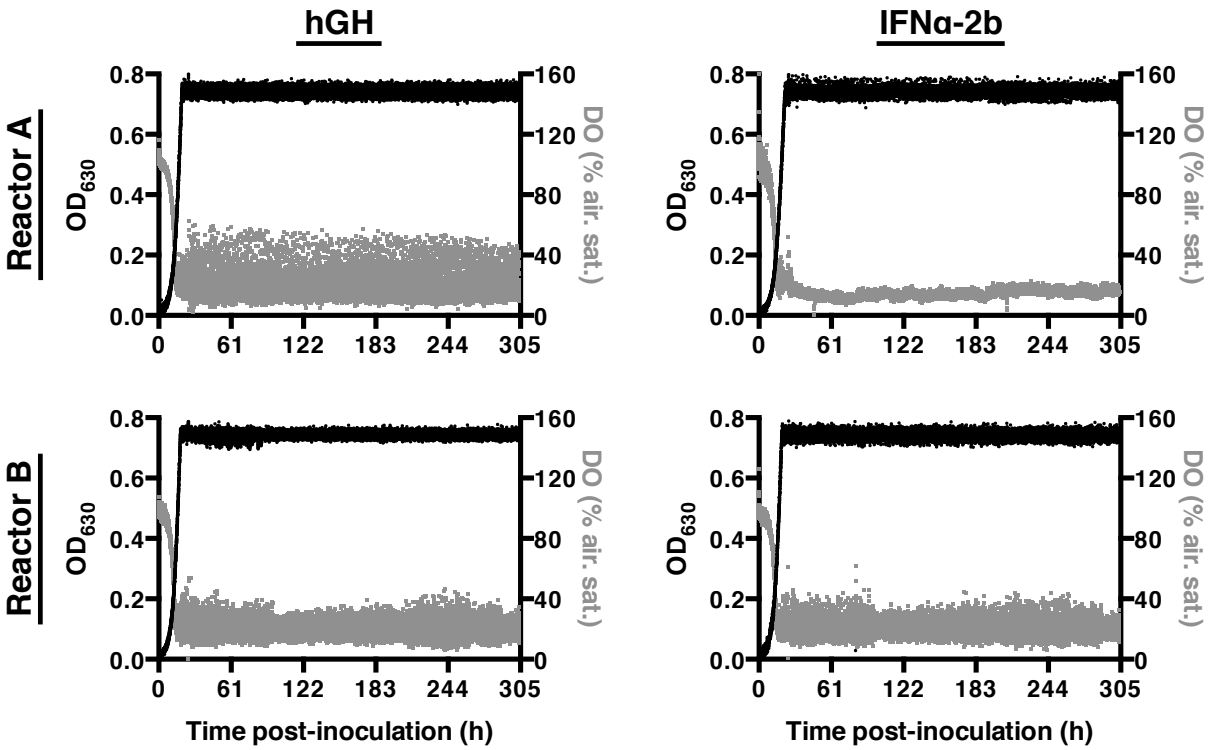

Fig. S3: Perfusostat fermentation sustained long-term culture stability. Replicate online OD_{630} (black) and DO (grey) profiles generated by parallel perfusostat cultures of hGH- and IFNα-2b-secreting strains of *P. pastoris*.

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