Supplementary information

Extraction, Enrichment, and *in situ* Electrochemical Detection on Lab-on-a-Disc; Monitoring the Production of a Bacterial Secondary Metabolite

Sune Zoëga Andreasen,[a†*] Kuldeep Sanger,[a†] Christian Bille Jendresen,[b] Alex Toftgaard Nielsen,[b] Jenny Emnéus,[a] Anja Boisen,[a] Kinga Zór,[a*]

Department of Micro- and Nanotechnology, Technical University of Denmark, 2800 Kgs. Lyngby, DENMARK
The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, 2800 Kgs. Lyngby, DENMARK.
† These authors contributed equaly to the work.
* Corresponding authors

Video showing the functionality of the fluidics

Video V1, shows with 8.5x increased speed, an extraction from an acidified (addition H_2SO_4) sample, where the nanoporous membrane was impregnated with dihexyl ether (DHE). The acceptor is filled with Tris buffer, pH 7.6. The acidified sample flows from the donor to the waste chamber, through the supported liquid membrane (SLM), where the extraction takes place.

Optimization of the SLM extraction

The static SLM extraction unit (Figure S1a) designed in a CAD software (AutoCAD, Autodesk, USA), consisted of an acceptor (1 mL) and donor chamber (2 mL) and the nanoporous polypropylene (PP) membrane (Celgard 2500, Celgard, LLC, Charlotte, NC, USA) sandwiched between the chambers. The electrochemical measurements were performed with CHI1030A potentiostat (CH Instruments (CHI), Inc., Austin, TX, USA) using commercial electrodes (DS220AT from Dropsens, Spain).
The donor chamber was designed to have two times larger volume than the acceptor, thus the expected maximum enrichment factor (EF) was 2, considering the ratio between donor and acceptor volume.[53] When optimizing the extraction conditions and choosing the organic phase, the acceptor buffer was phosphate buffered saline (PBS) (10mM PB, 150 mM NaCl) acidified (pH 2) with H_2SO_4. At pH 2 pHCA is neutral while Tyr is charged. As acceptor buffer PBS was used at pH 7.4, at this pH pHCA will be charged. To evaluate the efficiency of the SLM extraction n-undecane, DHE, and DHE with 5% trioctyl phosphine oxid (TOPO) were tested as organic phase. For each experiments, three different solutions were prepaired in acidified PBS and used for extraction namely, 1 mM pHCA, 2 mM Tyr and 1 mM pHCA+ 1mM Tyr. The SLM extraction unit filled with the donor and acceptor were left overnight (18 hours), for the extraction to take place, after which the acceptor was recovered and measured electrochemically (Figure S1b). Figure S1b shows the electrochemical signal recorded from Tyr, pHCA and mixture of Tyr and pHCA using square wave voltammetry (SWV) in PBS, pH 7.4 and

it can be observed that detection of pHCA in the presence of Tyr is cumbersom, due to the close proximity of their redox potentials. After SLM extraction we found that Tyr was not present in the acceptor, while a clear peak was recorded in the same region where pHCA was detected (Figure S1c). Figure S1d shows that the highest enrichment factor (EF) was obtained with the more polar oils, DHE and DHE with 5 % TOPO.

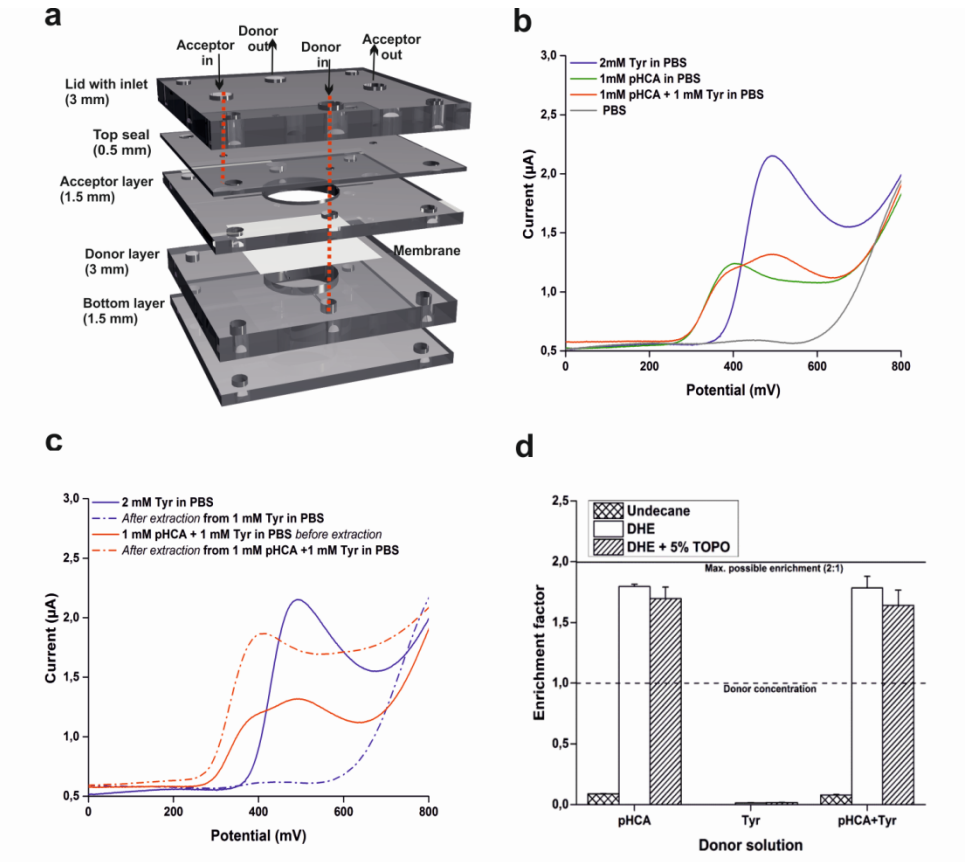

Figure S1: (a) Exploded view of the SLM extraction unit used for optimising the choice of the organic phase. All grey parts are poly(methyl methacrylate) PMMA and the membrane is a nanoporous polypropylene (Celgard 2500); (b) Square wave voltamogramm (SWV) recorded in PBS, pH 7.4; (c) SWV recorded before and after SLM extraction in PBS 7.4, using DHE as organic phase; (d) Comparing the three different oils (n-undecane, DHE, and DHE + 5% TOPO) in terms of EF after 18 hours of passive extraction. The maximum enrichment factor possible is given by the ratio of donor to acceptor volume in contact with the membrane, e.g. 2:1 in this case. Standard deviations is calculated from n=3 samples. Potential vs. Ag pseudo-reference electrode (pseudo-RE).

Electrochemical detection of pHCA in the presence and absence of Tyr in control cell culture supernatant.

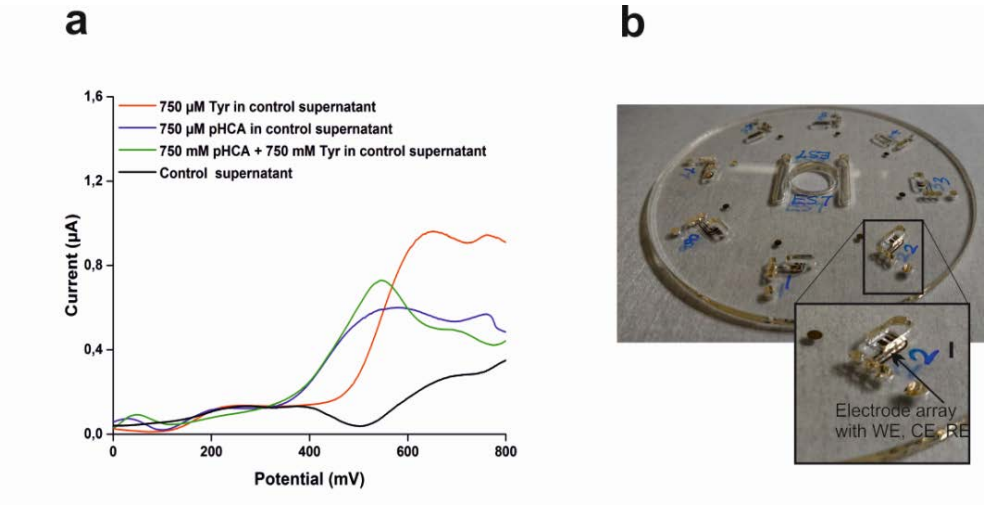

Figure S2. (a) SWV of Tyr, pHCA and mixture of the two analytes spiked the in control supernatant collected at 24h from non-pHCA producing bacteria, pH 5.8. Potential vs. Ag pseudo-reference electrode (pseudo-RE); (b) Photograph of the static 'open vial LoD system', used for measurement of the sample and controls before extraction as well as for the electrode characterization and pre-calibration. Inset shows the close up the electrochemical detection chamber with one of the three electrode array.

E. coli **growth and production of pHCA and consumption of Tyr during 24h of culture**

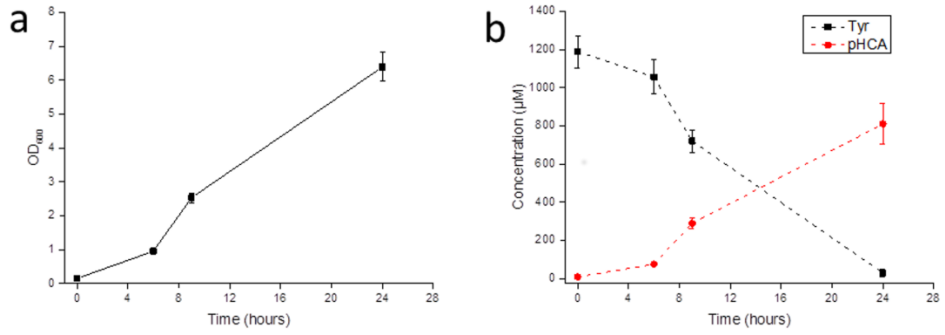

Figure S3: Representative data presenting bacterial cell growth (a) and HPLC data showing Tyr consumption and pHCA production during a 24 hour culture (b).

Chromatogramms obtained from samples before and after SLM extraction

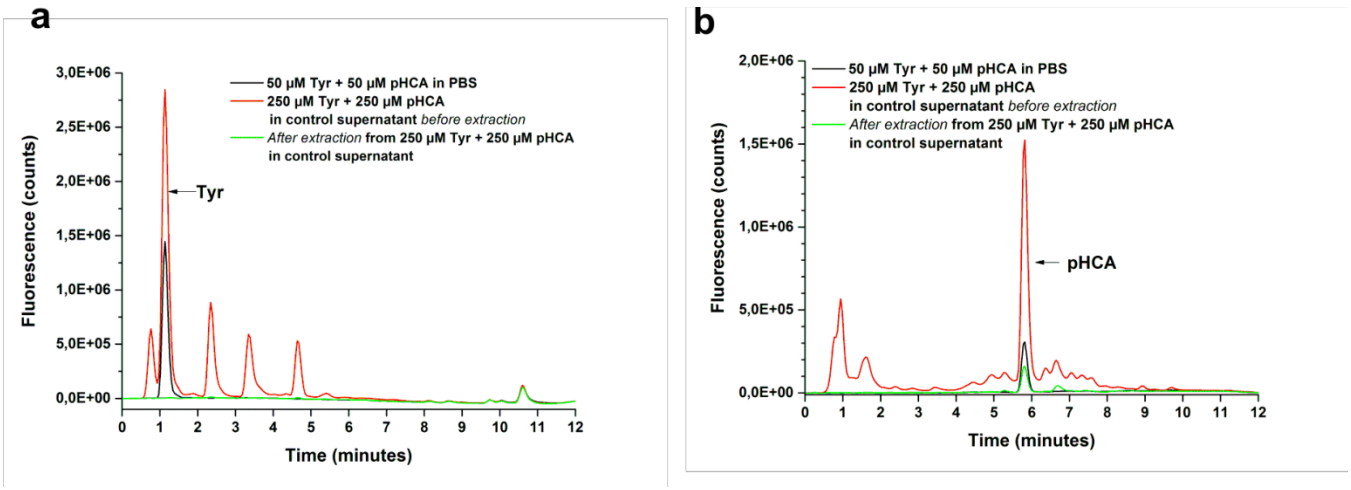

Figure S4: Chromatogramm obtained with parameters optimized for (a) Tyr and (b) pHCA detection. In both cases the black line is PBS spiked with 50 µM Tyr and 50 µM pHCA, red line represents sample (control supernatant with Tyr and pHCA) before extraction, while green line is the sample after 10 min extraction to the acceptor (Tris pH 7.6) buffer.

The data from HPLC (Figure S4) shows the efficiency of the SLM extraction. It can be observed that pHCA and Tyr is present in spiked PBS as well as in the sample before extraction at retention time of 5.7 min and 1.2 min respectively. After extraction there is no Tyr present in the acceptor buffer (Figure S4a), while pHCA can be clearly detected (Figure S4b). The signal intesity difference between 250 µM pHCA before and after extraction is due to the fact that the sample after SLM extraction was diluted 6-times to be able to obtain enough volume for HPLC analysis in triplicates.