**Article information**

**Article title:**

HEK293 producing the extracellular domain Her1: full datasets of continuous fermentation process and metabolites analysis

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**Keywords**

HEK293 cell line, continuous culture dataset, metabolic dataset, PCA analysis

**Abstract**

The data for provide evidences of the multi steady state of the human cell line HEK 293 was obtained from 2L bioreactor continuous culture. An HEK 293 cell line transfected to produce soluble HER1 receptor was used. The bioreactor was operated at three different dilution rates in sequential manner. Daily samples of culture broth were collected, a total of 85 samples were processed. Viable cell concentration and culture viability was addressing by trypan blue exclusion method using a hemocytometer. Heterologous Her1 supernatant concentration was quantified by a specific ELISA and the metabolites by mass spectrometry coupled to a liquid chromatography.

The primary data were collected in excel files, where it was calculated the kinetic and other variables by using mass balance and mathematical principles. It was compared the steady states behavior each other’s to find out the existence of steady states´ multiplicity, taking into account the stationary phase with respect to the cell density (which means its coefficient of variation is less than 20 %).

From the metabolic measurements by using Liquid Chromatography coupled to mass spectrometry (LC-MS), it was also built the data matrix with the specific rates of the 76 metabolites obtained. The data were processed and analyzed, using multivariate data analysis (MVDA) to reduce the complexity and to find the main patterns present in the data.

We describe also the full data of the metabolites not only for steady states but also in the time evolution, which could help others in terms of modelling and deep understanding of HEK293 metabolism, especially under different culture conditions.

**Specifications table**

|  |  |
| --- | --- |
| **Subject** | Biological science |
| **Specific subject area** | Cell culture biotechnology and metabolic profiling |
| **Type of data** | Table  Figure |
| **How the data were acquired** | The data were acquired from different sources listed below:  Viable cell density and viability, via optical microscope by trypan blue dye exclusion method. Samples were diluted in trypan blue depending on the expected cell concentration. The data were recorded at the electronic notebook.  Extracellular domain Her1 concentration, via microplate photometer by ELISA sandwich method (GEN5 Reader Control Software). The ELISA method was homemade developed. Briefly, it was used an antiHer1 antibody for plates´ coating and as a protein captor. Then, the samples from the culture are added to the plates. An anti-EGFr antibody conjugated with biotin is added, and Streptavidin-Peroxidase reagent is incorporated to the plates. Tetra-Methyl-Benzidine (TMB) is used as substrate for peroxidase. Later, plates are read and data is saved at the electronic notebook as well.  Metabolites concentration, via Liquid Chromatography-Mass spectrometry (LC-MS) by derivatization method (TraceFinder software). Metabolites are extracted from the samples by using an extraction buffer prepared with acetonitrile, methanol and deionized water, and later are transferred to LC-MS vials. Then, samples were measured at the LC-MS equipment, where metabolites are first separated by their retention time and later by their specific mass/charge (m/z). The LC-MS raw data is then preprocessed using TraceFinder software. |
| **Data format** | Raw  Analyzed  Filtered |
| **Description of data collection** | The data correspond to the fermentation process in continuous mode of HEK293 cells producing the heterologous glycoprotein Her1, in a 2L bioreactor.  Samples were taken daily from the culture for subsequent analysis of cell concentration, protein concentration and metabolites measurement.  The metabolic data were autoscaling-standardized (ratio of centered mean and the standard deviation) prior the analysis*.* |
| **Data source location** | Cells and protein concentrations:  · Institution: National Institute of Molecular Immunology. Center of Molecular Immunology  · City/Town/Region: La Habana  · Country: Cuba  · Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: https://goo.gl/maps/zECCtx6P2T1SuN2i6  Metabolite measurement:  · Institution: Beatson Institute for Cancer Research  · City/Town/Region: Glasgow  · Country: UK  · Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: https://goo.gl/maps/ePAPCNLz259M9WJf7 |
| **Data accessibility** | Pre-processed data is available at Mendeley Data [[1](#_ENREF_1)]  Data identification number: 10.17632/t9rcjv5362.2  Direct URL to data:[*https://data.mendeley.com/datasets/t9rcjv5362/1*](https://data.mendeley.com/datasets/t9rcjv5362/1) |
| **Related research article** | L. Calzadilla, E. Hernández, J. Dustet, J. Fernandez-de-Cossio-Diaz, K. Leon, M. Pietzke, A. Vazquez, R. Mulet, T. Boggiano, Multiple steady states and metabolic switches in continuous cultures of HEK293: Experimental evidences and metabolomics analysis. *Process Biochemistry*. *In Peer review process*. |

**Value of the data**

* There are no records about the multiplicity of steady state in a human cell line: HEK293, as far as we know. The experimental results were obtained in continuous mode, specifically with the same medium formulation. The switch from one state to the other was reached by manipulating the dilution rate.
* The information of 76 metabolites from different pathways are displayed in the data. Furthermore, we describe the time evolution of them. Hence, the community of theoretic science will find this information very useful.
* The data can be useful for the understanding the plasticity of HEK293 metabolism, specially, under different metabolic patterns. Strategies for process development emerge from the data analysis as growth and heterologous protein production are addressed.

1. **Data description**

HEK293 cell line, producing the extracellular domain Her1 (ECD-Her1), was cultured in continuous mode using a 2L bioreactor for 86 days. It was experimentally assayed different dilution rates (D) in order to obtain several steady states. Table 1 describes the data generated from the daily culture sampling, such as the culture time, the cell density, the viability, the ECD-Her1 concentration, the corresponding D and the zone where the steady states were defined. The full information of the rest of variables associated to the culture can be found at the repository.

On the other hand, from the daily samples, the measurement of 76 metabolites was carried out by using Liquid Chromatography-Mass Spectrometry (LC-MS), and table 2 collects the name of those measured metabolites. The specific rates of metabolites were calculated from the mass balance equations [[2](#_ENREF_2),[3](#_ENREF_3)], having the metabolites output measurement and the medium formulation. We create a data matrix, with the specific uptake or production rates of the metabolites and the steady states, organized in a variable wise mode, which can be found also at the repository. Multivariate data analysis (MVDA) approaches were spread over the matrix mentioned above, in order to find the main patterns in the data. With the aim of finding the correspondence of each metabolite with the main patterns in the data, we also compute the correlation loading graph, and figure 1 show the results. Full information of MVDA results can be found at the repository files.

1. **Experimental design, materials and methods**
   1. **Cell culture protocol**

In order to test the multiplicity of steady states in HEK293 cell line, cells were culture in continuous mode, keeping the same medium formulation (which can be found at the repository). Three D (0,35 d-1; 0,4 d-1 and 0,45 d-1) were assayed. The continuous operation was started at 0,45 d-1 followed by 0,4 d-1 and later was reduced down to 0,35 d-1. Then, D was progressively increased to 0,4 d-1 and later 0,45 d-1 once more. Each steady state was kept for at least four days after the stabilization (which means coefficient of variation of cell density less than 20 %).

Samples were taken from the culture on daily base to quantify cell concentration, viability, ECD-Her1 concentration and metabolites concentration.

* 1. **Quantification methods**
* Cell density and viability were determined by trypan blue dye exclusion method in Neubauer chamber and using optical microscopy. It was applied the sample dilution in trypan blue depending on the expected cell density.
* The ECD-Her1 concentration was determined by a homemade ELISA sandwich method. 96-microwell plates were coated with 5 mg/mL of an anti-Her1 (Center of Molecular Immunology propriety), at least the day prior to the assay and kept at 4 °C. At the moment of the assay, samples and standard are bringing to room temperature, and serial dilutions are applied to them in order to obtain the samples concentration in the curve range. Then, they are added to the plates and incubated at 37 °C 1 h. Three automated washing step is applied, and then an anti-EGFr antibody conjugated with biotin (R&D system) is added to the plate and incubated at 37 °C 1h. Three automated washing step is applied again, and Streptavidin-Peroxidase reagent (R&D system) is added to the plate and incubated at 37 °C 1 h. The plates are automated washed for three times. Finally, TMB substrate (R&D system) is added to the plate. After 20 minutes the reaction is stopped with sulfuric acid and the plates are read by a spectrophotometer at 450 nm.
* The metabolites measurement was carried out by LC-MS technique. Metabolites are primarily extracted from the samples, that are diluted 1:100 in extraction solvent, LC-MS grade (ACN:MeOH:H2O, 3:5:2) [[4](#_ENREF_4)]. Then, diluted samples are well mixed in vortex for 20 s and centrifuged at 14000 g x 10 min and 4 °C. At that point, samples are transferred to LC-MS vials for separation and detection in the LC-MS equipment. Metabolites are separated by the LC column ZIC-pHILIC column (Merck Millipore) with depend of their retention times, using metabolites´ standards from Sigma Aldrich (Merck Millipore). For the separation it is used 20 mM ammonium carbonate as aqueous mobile phase solvent, adjusted to pH 9.4 with 0.1 % ammonium hydroxide solution (25 %); and 100 % Acetonitrile as organic mobile phase. It is applied a lineal gradient strategy at 200 mL/min for the separation process that take around 15 minutes, and followed by an equilibration step. Column is kept in the oven at 45 °C, and samples are maintained at 4 °C prior to injection to the Q-Exactive mass spectrometer with auto-sampler, Heater Electro Spray Ionization source (HESI), and ORBITRAP as detector (AGILENT). Finally, the metabolites are separated by their mass/charge (m/z) with a mass accuracy below to 5 ppm with the Q-Exactive mass spectrometer.

**Ethics statements**

This work does not include data from human subjects, animal experiments or data collected from social media platforms.

**CRediT author statement**

**Lisandra Calzadilla:** Methodology, Investigation, Formal analysis, Data curation, Writing-original draft, Visualization. **Erick Hernández:** Formal analysis, Data curation. **Matthias Pietzke:** Resources. **Alexei Vazquez:** Resources, Supervision, Funding acquisition. **Julio Dustet:** Supervision. **Jorge Fernandez-de-Cossio-Diazand Kalet León:** Creation of model. **Roberto Mulet and Tammy Boggiano:** Conceptualization, Methodology, Supervision, Writing-review & editing. All authors contributed by reading, edition and approval the final manuscript.

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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