Monitoring of antibody glycosylation pattern based on microarray MALDI-TOF mass spectrometry

Author(s):
Hajduk, Joanna; Wolf, Moritz; Steinhoff, Robert; Karst, Daniel; Souquet, Jonathan; Broly, Hervé; Morbidelli, Massimo; Zenobi, Renato

Publication date:
2019-08-20

Permanent link:
https://doi.org/10.3929/ethz-b-000351707

Originally published in:
Journal of Biotechnology 302, https://doi.org/10.1016/j.jbiotec.2019.06.306

Table S1. mAb quantification based on Protein A HPLC-UV analysis. Abbreviations: WD – working day, VCD – viable cell density.

WD	VCD [10^6 cells/day]	Titer [g/L]
1	38.453	0.379
2	39.113	0.471
3	39.947	0.518
4	36.994	0.472
5	39.495	0.470
6	38.384	0.439
7	39.634	0.430
8	40.034	0.308
9	39.582	0.364
10	39.426	0.351
11	39.704	0.397
12	46.964	0.390
13	49.370	0.375
14	49.971	0.411
15	51.371	0.340
16	52.972	0.289
17	49.646	0.308
18	50.571	0.299

Perfusion cell culture conditions:

A stable culture at both viable cell density set points of 40 and 50x10^6 cells/mL was achieved over the entire 18-day run. The cell viability remained above 90%. The extracellular glucose level varied between 16.1 and 24.6 mmol/L during the first set point, and 14.4 and 19.6 mmol/L during the second cell density set point. Lactate concentrations ranged between 11.1 and 17.8 mmol/L, and between 6.9 and 10.7 mmol/L, respectively. After an initial transition period resulting from cell inoculation to the perfusion bioreactor, as well as the change of the cell density set point, Glc (20.8, and 16.2 mmol/L) and Lac (14.2, and 6.6 mmol/L) concentrations approached constant levels during each of the steady states.

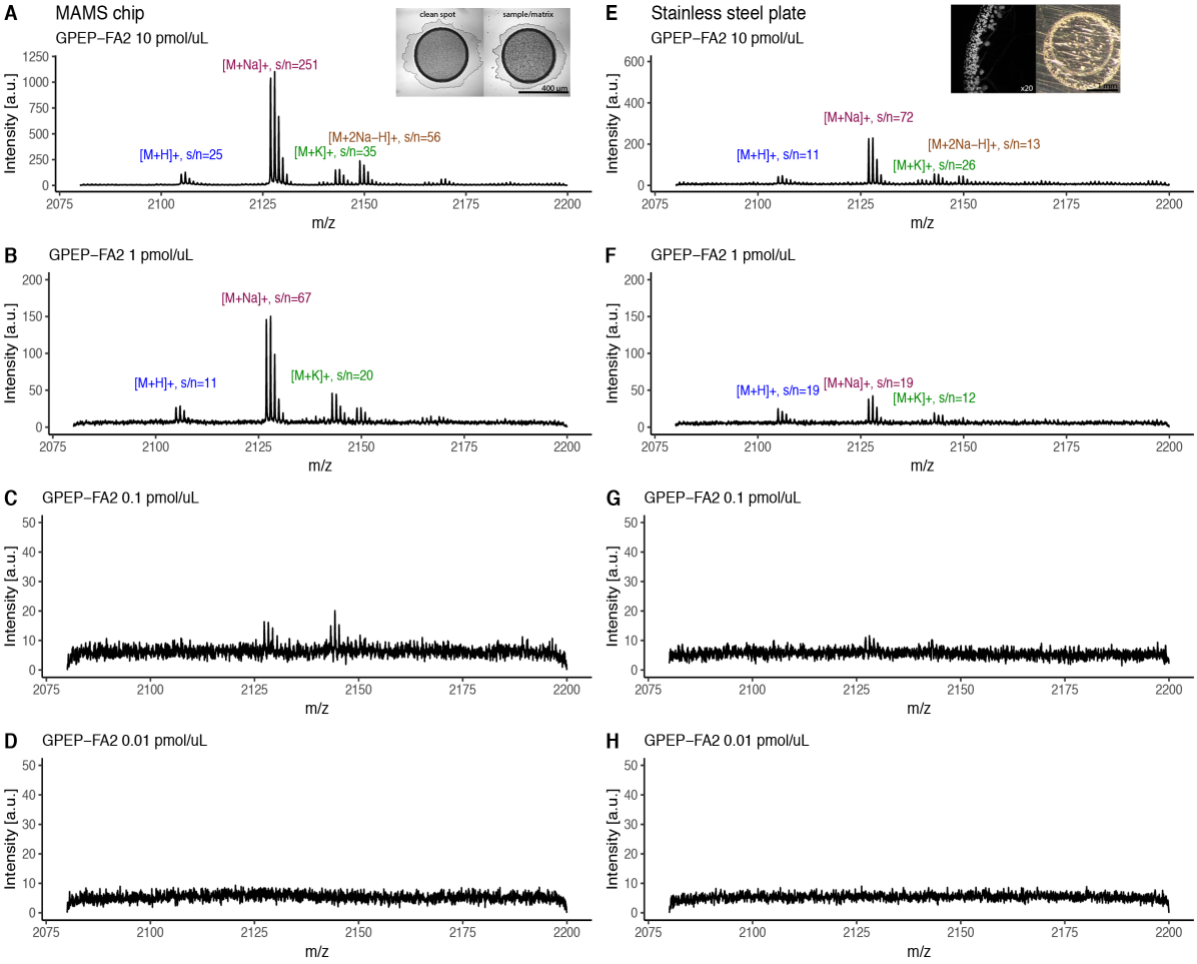

Figure S1. MALDI-TOF MS spectra of standard FA2 glycopeptide in the concentration range from 10 to 0.01 pmol/µL. MAMS sample deposition (A-D) vs. conventional deposition on a stainless-steel target (E-H). The recorded m/z: 2104.93 $(M+H)^+$; 2126.92 $(M+Na)^+$, 2142.88 $(M+K)^+$, 2148.89 $(M+Na-H)^+$. The signal-to-noise ratios (s/n) are plotted for the major peaks, and was found to be 3.5 times higher for the MAMS samples. The FA2 glycopeptide amino acid sequence: KVANKT.

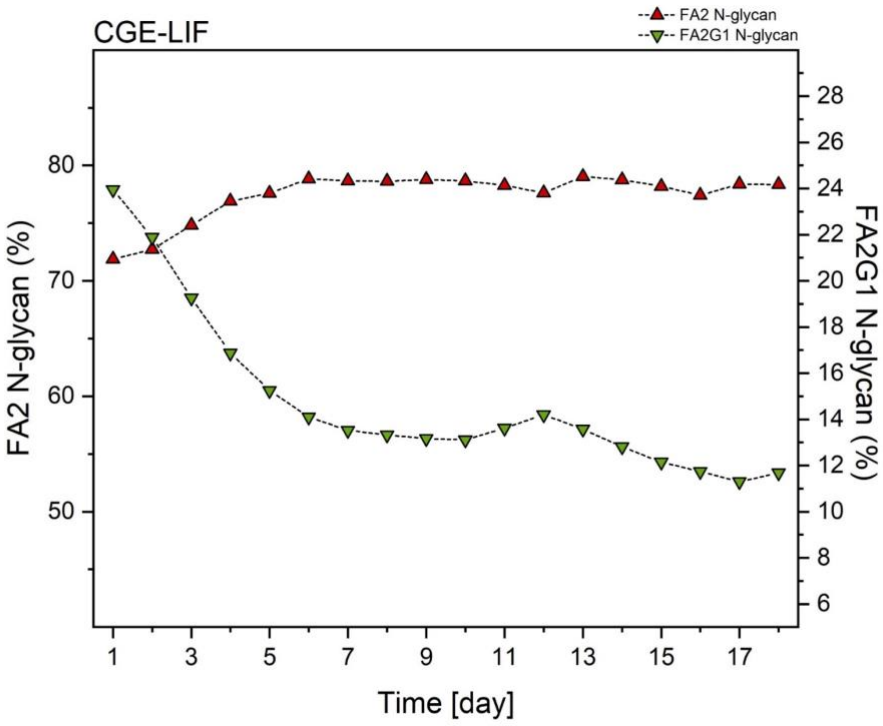

Figure S2. CGE-LIF reference analysis of FA2 (red triangular, dotted line) and FA2G1(green triangular, dotted line) N-glycan structure.

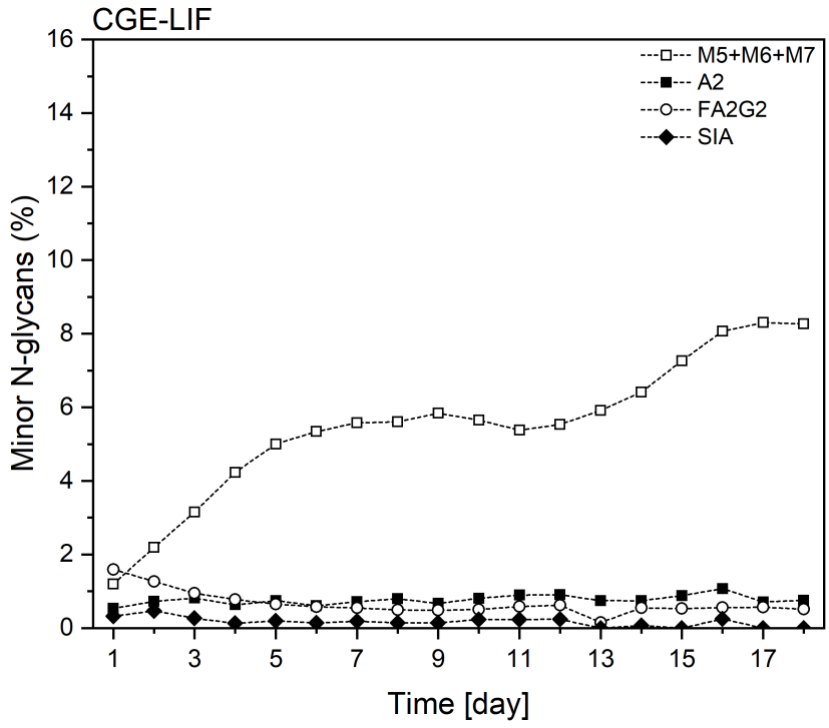

Figure S3. CGE-LIF reference analysis of N-glycan's minor fraction: high mannose (M5+M6+M7; open square, dotted line), A2 (black square, dotted line), FA2G2 (open circle, dotted line) and sialylated forms (SIA; black diamond, dotted line).

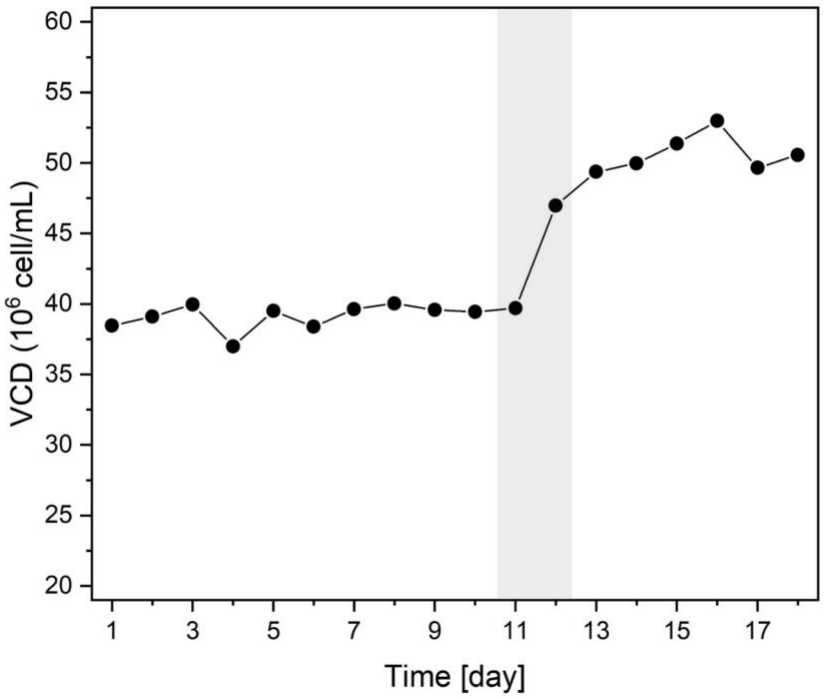

Figure S4. Viable cell density (VCD) as a function of time.