



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

## Journal Article

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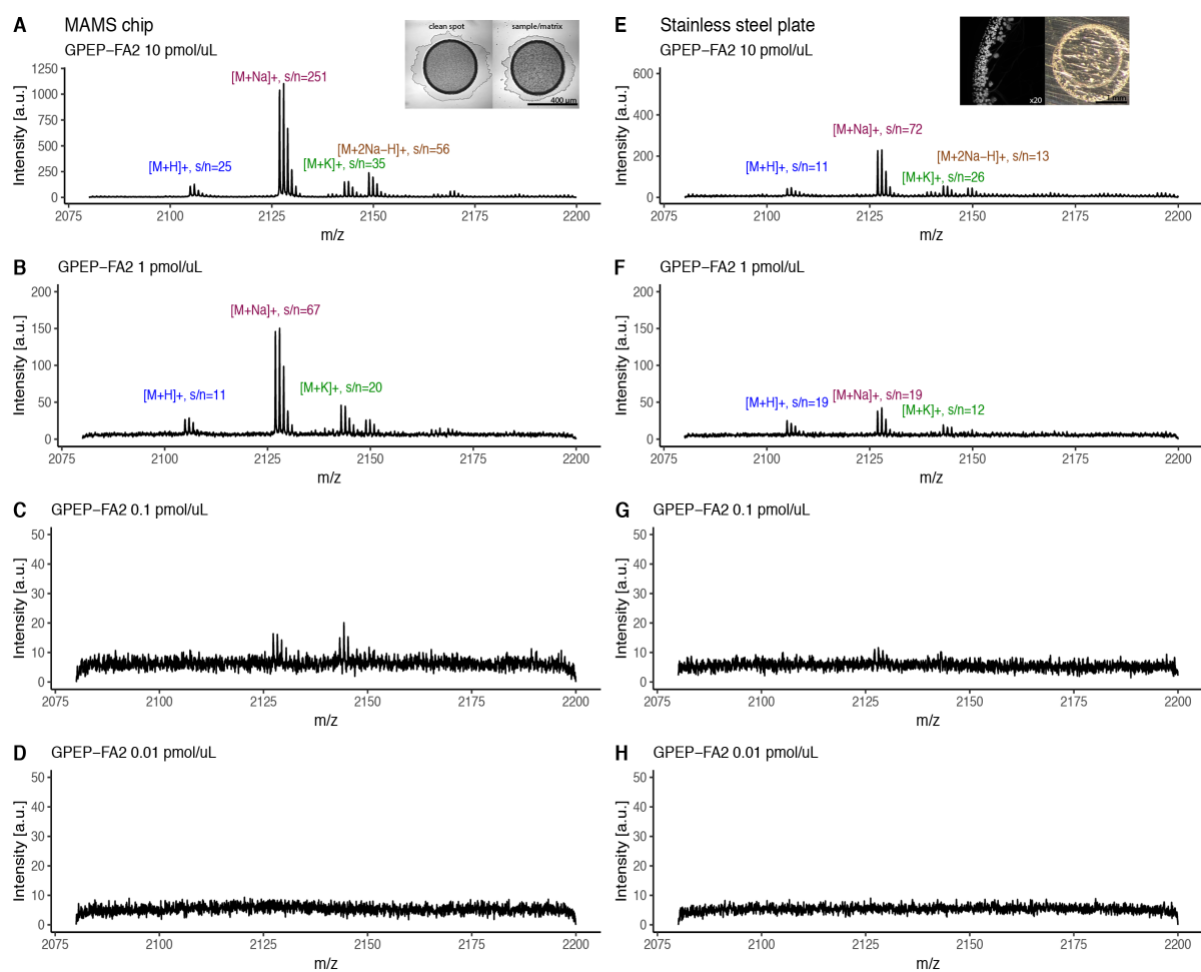
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**Table S1.** mAb quantification based on Protein A HPLC-UV analysis. Abbreviations: WD – working day, VCD – viable cell density.

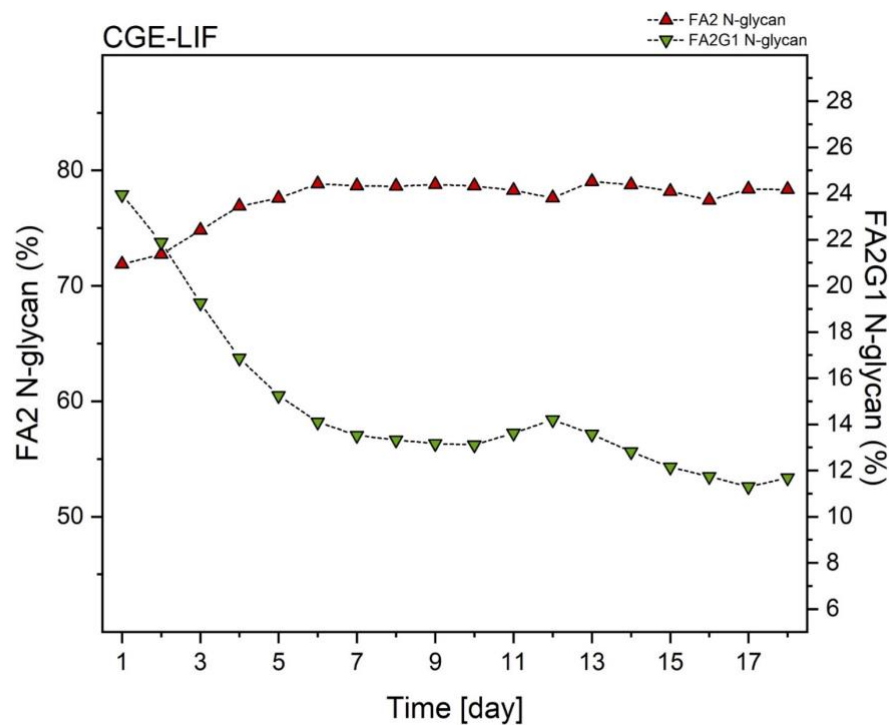
WD	VCD [ $10^6$ cells/day]	Titer [g/L]
1	38.453	0.379
2	39.113	0.471
3	39.947	0.518
4	36.994	0.472
5	39.495	0.470
6	38.384	0.439
7	39.634	0.430
8	40.034	0.308
9	39.582	0.364
10	39.426	0.351
11	39.704	0.397
12	46.964	0.390
13	49.370	0.375
14	49.971	0.411
15	51.371	0.340
16	52.972	0.289
17	49.646	0.308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

**Perfusion cell culture conditions:**

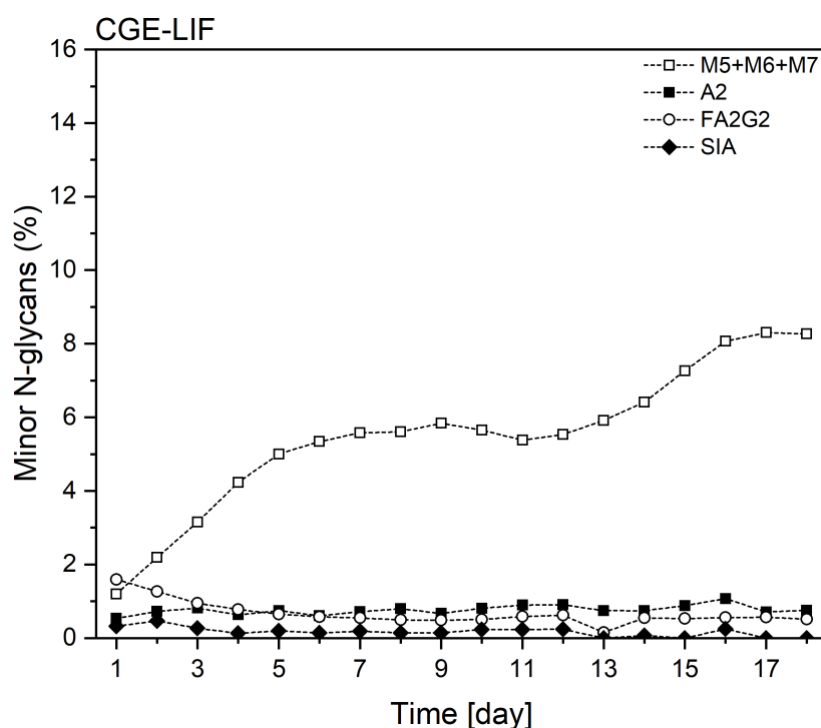
A stable culture at both viable cell density set points of 40 and 50x10<sup>6</sup> cells/mL was achieved over the entire 18-day run. The cell viability remained above 90%. The extracellular glucose level varied between 16.1 and 24.6 mmol/L during the first set point, and 14.4 and 19.6 mmol/L during 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.



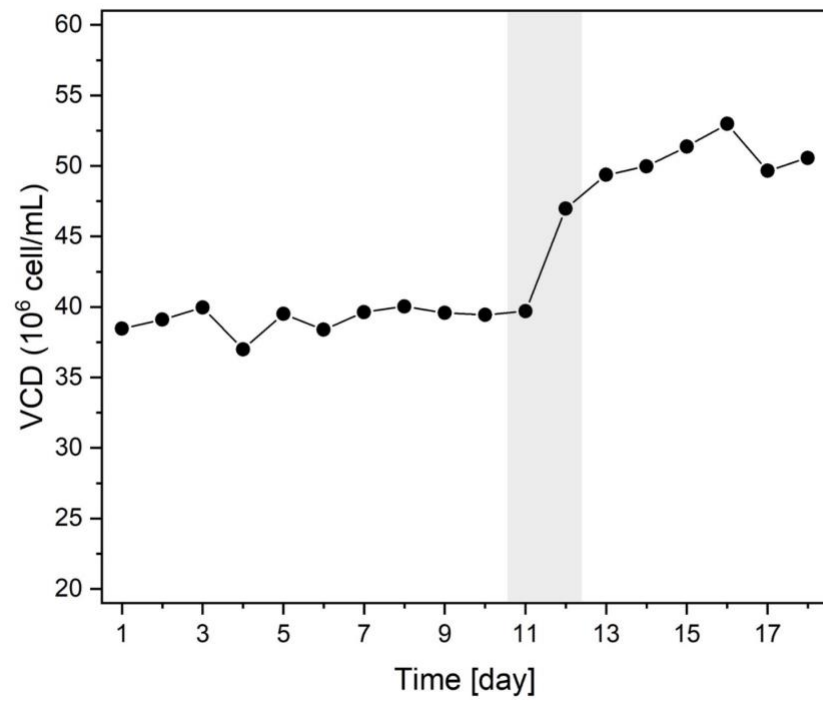
**Figure S1.** MALDI-TOF MS spectra of standard FA2 glycopeptide in the concentration range from 10 to 0.01 pmol/μL. MAMS sample deposition (A-D) vs. conventional deposition on a stainless-steel target (E-H). The recorded m/z: 2104.93 ( $[M+H]^+$ ), 2126.92 ( $[M+Na]^+$ ), 2142.88 ( $[M+K]^+$ ), 2148.89 ( $[M+Na-H]^+$ ). 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.