Supplementary Information (SI)

## Automated Instant Labeling Chemistry Workflow for Real-Time Monitoring of Monoclonal Antibody N-Glycosylation

Aron Gyorgypal<sup>a,b</sup>, Oscar Potter<sup>c</sup>, Antash Chaturvedi<sup>a</sup>, David N. Powers<sup>b</sup>, Shishir P. S. Chundawat<sup>a\*</sup>

<sup>a</sup> Department of Chemical and Biochemical Engineering, Rutgers University, Piscataway, New Jersey, USA <sup>b</sup> Center for Drug Evaluation and Research, Office of Product Quality, Office of Biotechnology Products, Division of Biotechnology Review and Research II, U.S. Food and Drug Administration (FDA), Silver Spring, Maryland, USA <sup>c</sup> Agilent Technologies, Inc., 5301 Stevens Creek Blvd Santa Clara, California, USA

\*Corresponding Author: Shishir P. S. Chundawat (shishir.chundawat@rutgers.edu)

## Supplementary figures legend

**Supplementary Fig. 1.** Sensitivity analysis of Trastuzumab glycosylation using varying concentrations of mAb for analysis. The figure shoes the fluorescence intensity peak areas as a function of concentration between a low limit of detection from mAb at 0.05 g/L to upwards of 4.0 g/L. Samples were prepared from the same 4 g/L stock making dilutions with 50mM HEPES to reach each desired concentration prior to the IPC Kit chemistry sample preparation and analysis. Relative quantitation is possible at the lower concentrations using IPC chemistry.

**Supplementary Fig. 2.** FLR chromatography of VRC01 neutralizing antibody sample from offline HPLC analysis on LC-MS system but prepared using proposed N-GLYcanyzer system with InstantPC labeling workflow. Proof of concept results that showcase how our automated N-Glycanyzer sample preparation workflow allows for sialylated glycoforms detection. Peaks were identified based on MS analysis as described within the main text. Residence times are different as samples were run on different LC systems.



Supplementary Fig. 1.

