A Cost Effective Measurement Device for Quantifying Antibodies Containing Glycans Using Lectin Binding

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Summary

The purpose of this study is to develop a biosensor using quartz crystal microbalance (QCM) technology which will allow for cost effective measurement of glycan bound antibodies for future glycan modification protocols. The biosensor will utilize “calibration free” microfluidic technology which will provide similarly accurate results as more expensive measurement technologies.

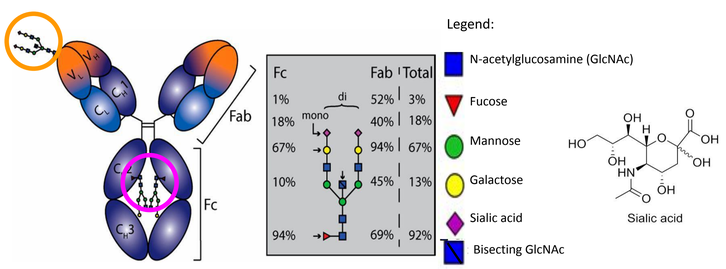
OBJECTIVES:

- Biosensor stability and optimization
- Biosensor sensitivity for SNA lectin binding to sialic acid
- Biosensor sensitivity for ECL lectin binding to galactose following removal of Fab glycan sialic acid with Neuraminidase

This sensor uses quartz microcrystal technology.

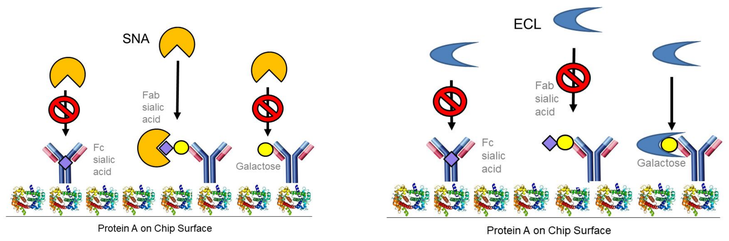
Fab glycans
14% of IgG have them.
Highly processed (long).
Fab sialic acid is easy to detect in IgG (exposed).
PNGase F cannot cleave unless IgG is “denatured”.

Fc glycans
All IgG have 2.
Shorter.
Fc sialic acid cannot be detected in intact IgG.
PNGase F will always cleave.



(S with HO or “g” indicates glycolated Sialic acid)

The system uses an automatic pump to control the flow rate (mL/hour) of the solutions being added.



As molecules bind to the surface of the sensor, the frequency of the vibrations change. These frequency changes are used to determine the amount of bound antibodies present in a sample.

Methods and Materials

The following substances are added to the system:

- Copper (II) ions
- Antibodies (IgG)
- ECL or SNA Lectins

Between each addition, a phosphate-buffered solution (PBS) was added to maintain a biologically appropriate pH for the system.

At the end of each cycle, the sensor was cleaned (Regen) using both glycerin (pH 1.5) and a stripping buffer. Copper was also reapplied to the sensor following each cleaning.

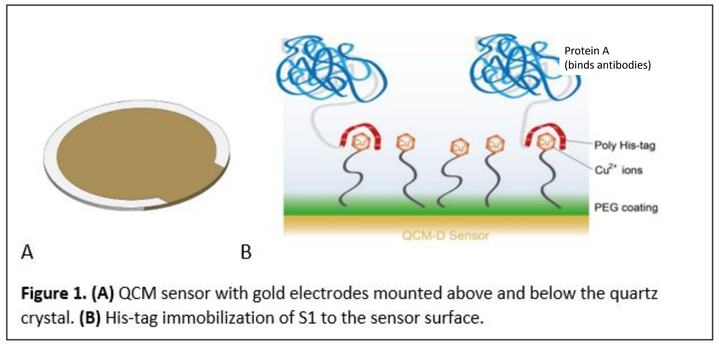


Figure 1. (A) QCM sensor with gold electrodes mounted above and below the quartz crystal. **(B)** His-tag immobilization of S1 to the sensor surface.

The frequency of oscillations of the sensor is measured continuously in hertz (Hz), producing a graph as seen in Figure 1.

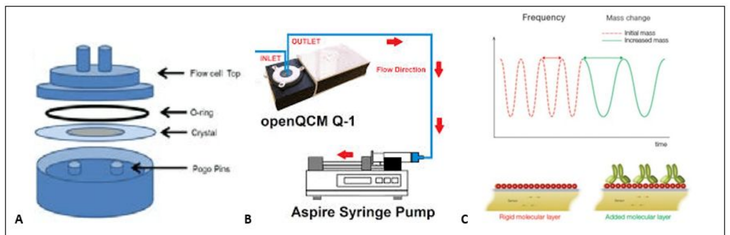


Figure 2. (A) Placement of QCM sensor in the flow cell. **(B)** Bioensor Flow System. **(C)** IgG binding causes a decrease in quartz crystal oscillation frequency proportional to mass added.

Three methods were used to detect 4G8 antibodies binding to Protein A as well as Lectin binding to Fab glycans.

Data was analyzed in several different ways

- The binding rate/slope was determined for an interval during the sample application
- The overall change in frequency during sample application was found
- The overall change in frequency during the SpA application was found

Results

- Significant binding to 4G8 antibody
- Protein A w/ His Tag showed significant binding w/ Neuraminidase treatment.
- Significant binding to SNA in both untreated and following initial Neuraminidase treatment and Neuraminidase with citrate.
- No significant binding to ECL lectin in either treated samples. Unclear results for ECL binding following Neuraminidase treatment.

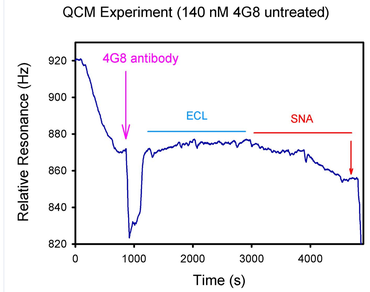


Figure 3. This graph shows the relative resonance response of sensor binding for 4G8 antibody, ECL, and SNA lectins without neuraminidase treatment.

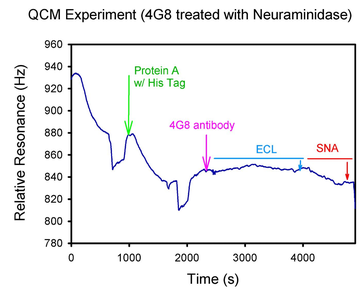


Figure 4. This graph shows the relative resonance response of sensor binding for 4G8 antibody, ECL, and SNA lectins with neuraminidase treatment as well as initial binding of protein A w/ Histidine tag to promote antibody binding.

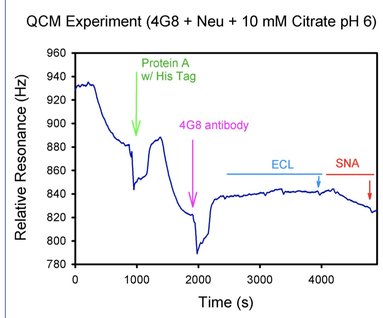


Figure 5. This graph shows the relative resonance response of sensor binding for 4G8 antibody, ECL, and SNA lectins with neuraminidase treatment as well as initial binding of protein A w/ Histidine tag to promote antibody binding.

CONCLUSION:

- SNA binding likely due to either degradation of neuraminidase or alternate lectin binding sites.
- Lack of ECL binding could be due to degradation of the ECL or the method in which the ECL was applied to the sensor.
- Further experimentation needed to determine expected binding sensitivity of lectins based on relative resonance impact due to size/weight disparities and available glycan binding sites.

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