Real-time bioanalysis of IgG-protein G interaction via quartz crystal microbalance

Stephan Drothler

I. INTRODUCTION

Quartz Crystal Microbalance (QCM) is a highly sensitive tool used to detect small mass adsorptions or medium density based on the inverse piezoelectric effect. Thereby a small voltage is applied in order to oscillate the crystal at its resonance (f_0) or higher order ($n \times f_0$) frequency. This technique has proven to be efficient at analyzing biological systems in real-time. The changes in oscillating frequency caused by analyte adsorptions are described in the Sauerbrey equation (1), where p_q (Density)$_{Quartz}$ = (2.643 $\frac{g}{m^3}$), μ_q (Shear modulus)$_{Quartz}$ = ($2.947 * 10^{11} \frac{g}{cm \times s^2}$), A (Active area)$_{Quartz}$, n (Order of Oscillation) and f_0 (Resonance frequency)$_{Quartz}$, are constant and can be inversely substituted for C (Kasper et al. 2016). Hence the changes in mass (Δm) can be calculated from equation (2), when measuring the shifts in frequency (Δf).

$$\Delta f = -\frac{2 \times n \times {f_0}^2}{\sqrt{p_q \times \mu_q}} \times \frac{\Delta m}{A} \tag{1}$$

$$\Delta m = -C \times \Delta f \tag{2}$$

The density(p_l) and viscosity(η_l) of a liquid strongly interfere with the oscillation of the quartz crystal and is described by the Kanazawa & Gordon Equation (3).

$$\Delta f = -{f_0}^{\frac{3}{2}} \times \sqrt{\frac{\eta_l \times p_l}{\pi \times p_q \times \mu_q}} \tag{3}$$

In the following experiment the density changes of a NaCl-dH_2O dilution series and the binding constant (K_D) of protein G with IgG antibodies were tried to be measured..

II. METHODS AND MATERIALS

A. MATERIALS

1) Tools:

- tweezers
- eppendorff-pipettes (10 μL, 200 μL, 1000 μL)
- greiner-tubes (12 mL)
- QCM setup (In-house construction)
- analyse balance
- eppendorf tubes (1.5 mL)
- quartz crystal (AT-cut, Area 20.47 mm^2)

2) Chemicals:

- avidin (de-glycolslated, ≈60 kDa)
- IgG (biotin tagged, ≈150 kDa)
- protein G
- PBS (150 mM NaCl, 15 mM NaH2PO4, pH 7.4)
- mQ-dH_2O
- NaCl

B. METHODS

1) Chip and chamber preparation:

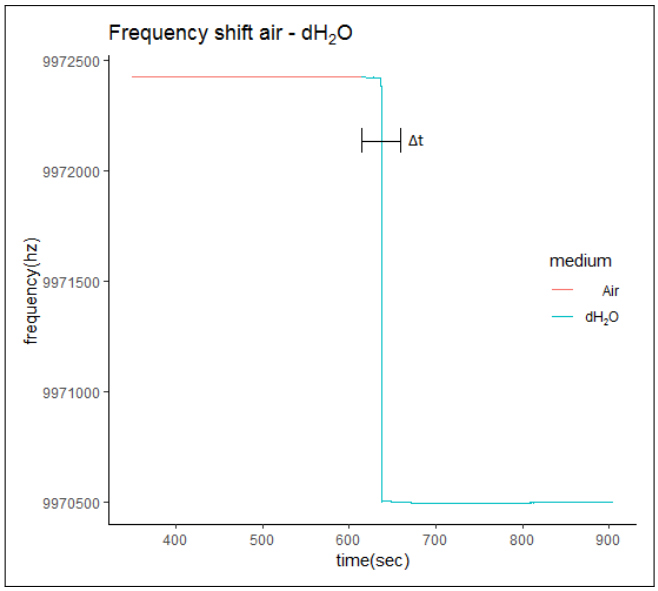
Chips were cleaned beforehand from the supervisor with a basic piranha solution. The ultra-clean chips were stored in ethanol and removed shortly before use. The liquid chamber was assembled by the supervisor and mounted to the QCM setup. The data was monitored on a self-written software (Keysight) based on MATLAB and analyzed using Rstudio (V 1.2.1335 R Core Team 2019). The plots were generated using the ggplot2 package (Wickham 2016).

2) NaCl dilution series:

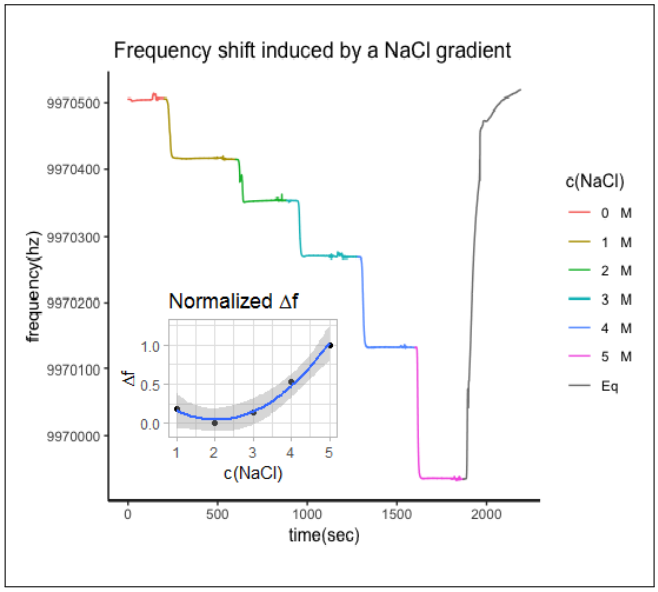
The liquid chamber of the QCM setup was first calibrated to excite the crystal at it's resonance frequency of 997243 Hz (Impedance = 135 Ω, n = 1). The quartz is allowed to settle stably at the frequency. while the dilution series is prepared. 5 × 1 ml NaCl-dH_2O solutions with the concentrations 1M, 2M, 3M, 4M and 5M were prepared. The flow-rate of 50 $\frac{\mu l}{min}$ and the average filling time (Δ t) of 85 sec resulted in a calculated dead volume of 70.5 μl (Demonstrated in Fig. 1a). The dead volume represents the volume needed to fill the chamber before stable data can be aquired. To ensure enough liquid reserve we chose volumes of 3-4 times the dead volume.

3) G protein - IgG K_D:

The chamber was first flooded with dH_2O, then with PBS in order to wash out residual NaCl and guarantee a stable oscillation. Afterwards the chamber was filled with the avidin solution and incubated for ≈10

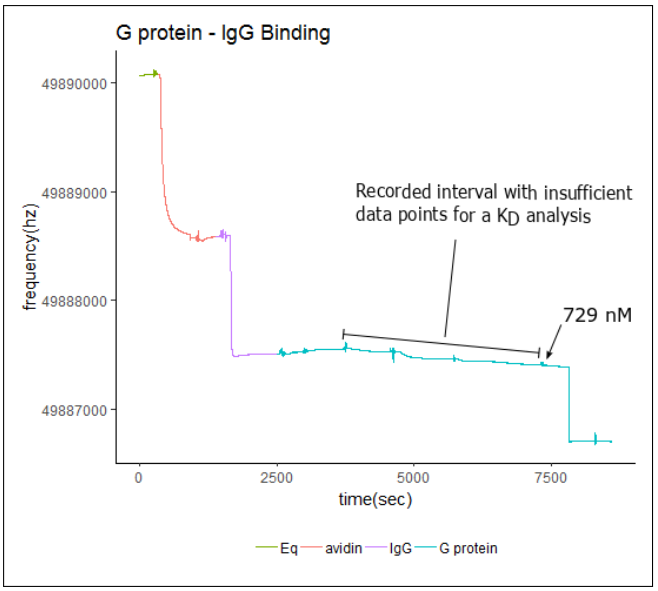


(a) Visualization of the frequency shift induced by a much denser medium

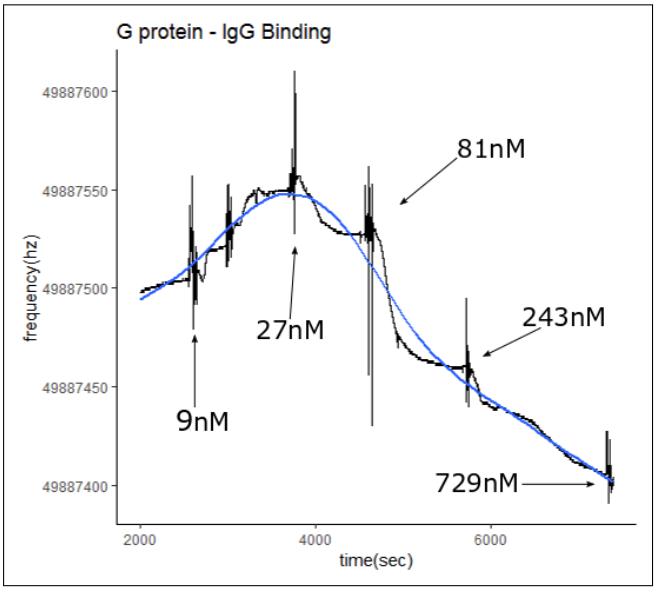


(b) Gradient tracking via Δf in the big plot. The small subplot shows the normalized Δf over the concentration shifts.

Fig. 1: Demonstration of frequency correlation with density and viscosity of the surrounding medium.



(a) Documentation of the IgG - G protein binding experiment, with avidin incubation (red), IgG injection (purple) and the G protein gradient (cyan).



(b) Higher resolution of the protein G gradient.

Fig. 2: Real-time tracking of adsorbent bio-molecules onto the gold coated layer of the quartz-crystal. The shifts in Frequency correlate thereby inversely with the total adherent mass (Eq. 1).

minutes. Thereby full saturation of the chip can be achieved. Based on the dead volume, solution volumes were calculated to 300 μL and accordingly prepared. The chamber was flooded with PBS in order to wash out residual or loosely bound avidin. Thereafter the biotinylated IgG antibody was pumped into the system. In this mode of operation, a higher order frequency of n = 5 was chosen and the protein G injections were prepared on a 3× dilution series (9 nM, 27 nM, 81 nM, 243 nM, 729 nM).

III. Results

Air to water frequency shift has been documented (Fig. 1a) and calculated (Δf = -1931 Hz). This value correlates well with the theoretical value Δf = -1900.333451 Hz calculated by the Kanazawa & Gordan equation (Eq. 3) ($p_l = 0.9980$ $\frac{g}{cm^{-3}}$,$\eta_l = 0.8928$ $\frac{mPa}{s}$)(Hai-Lang and Shi-Jun 1996, 0.0234 $\frac{mol}{kg}$ NaCl). The time gap for the dead volume of the QCM setup are also demonstrated in Fig. 1a.

Δ c(NaCl)	Δf	$\Delta f(Eq3)$	$\Delta f(Norm)$
0-1M	-89	-123.6	0.1879699
1-2M	-64	-138.9	0.0000000
2-3M	-83	-147.3	0.1428571
3-4M	-135	-228.3	0.5338346
4-5M	-197	-141.7	1.0000000

TABLE I: Frequency shifts for different c(NaCl) transitions

Furthermore, the frequency shift for the various NaCl concentrations was plotted (Fig. 1b) and the respective Δf values calculated (Tab. I/Δf). The Table also shows the theoretical shifts in frequency derived from the Kanazawa & Gordan equation (Tab. I/$\Delta f(Eq.3)$)(Hai-Lang and Shi-Jun 1996). The normalized Δf are derived from the measured values and also plotted in a subplot within Figure 1b. The measured frequencies appear to be lower in all cases, except 4-5M NaCl, than the calculated values.

The frequency plot for the IgG - G protein assay described in Methods are displayed in Figure 2a and further resolved in Figure 2b. Due to delayed exchange of 243 nM with the 729 nM solution, air bubbles formed and disturbed the residual measurement (Fig. 2a, $\approx$ 7700 sec). The K_D value is usually derived from the sigmaoid curve given by the normalized frequency shift over the protein G concentration. Due to an air bubble formation in the chamber, values after 729 nM injection, could not been recorded. Additionally, the first data point (1nM) distorts the remaining values. Thereby no K_D can be determined with significant confidence. The respective mass changes are calculated from equation 2 with C = 7.258×10^{10}. The resulting changes for avidin are 162.874 ng which is 25 % less than the reference of 218 ng. IgG binding resulted in a mass change of 84.97 ng which lies $\approx$ 52 % lower than the literature data of 177 ng Kasper et al. 2016).

IV. Discussion

The measured frequency decrease from air to water corresponds well to the theoretical values: Δf = -1931 Hz , $\Delta f_{\text{Theo.}}$ = -1900.3 Hz (Deviation $\approx$ 1.6 %). The theoretical value is based on a small concentration of salts (0.0234 $\frac{mol}{kg}$ NaCl) left in the H_2O and might therefore be slightly off. The value was chosen in order to provide a consistent basis of reference for the NaCl dilution series. The obtained values for the dilution series are below the academic notion ($\emptyset$ Deviation $\approx$ -41.1 %) with the exception of 4-5M NaCl (Deviation $\approx$ + 39 %). This deviation can partly be explained with the inaccuracy of the literature (5M = 4.9288M, 4M = 4.2179, 3M = 2.9405, 2M = 2.0010 1M = 1.0034). Temperature is also a variable which influences the measuring process and might have not been stable at 298.15 K (Hai-Lang and Shi-Jun 1996).

The relative mass changes of avidin and IgG accumulation is 25 and 52 % lower than expected (Kasper et al. 2016). This might also be due to incomparable room temperatures or partly because adsorption was not fully stabilized at the given incubation time (Fig. 2a). The value of higher order frequency is also not noted in the literature and might be higher than in the performed experiment which correlated with higher detection sensitivity. Because avidin was incubated first at a lower efficiency (- 25%) the high deviation of 52 % can be explained cumulatively. There was less surface for IgG to bind to, hence the deviation appears to be larger and amounts to - 27 % after recalculation. In an effort to deduce K_D the experiment should be re-performed with higher incubation times, careful observation of bubble formation and temperature, as well as appropriate exchange of the nano-molar G protein solutions. This might also lead to more precise avidin - IgG adhesion to the quartz surface.

V. Appendix

The experiment was performed at Gruberstrae 40, Linz, Austria on 16.05.19 under the supervision of Sabrina Meindlhumer. The experiment was carried out by Caroline Rieser, Lukas Schartel and Stephan Drothler.

References

[HS96] Zhang Hai-Lang and Han Shi-Jun. "Viscosity and density of water+ sodium chloride+ potassium chloride solutions at 298.15 K". In: *Journal of Chemical & Engineering Data* 41.3 (1996), pp. 516–520.

[Kas+16] Manuel Kasper et al. "Broadband 120 MHz impedance quartz crystal microbalance (QCM) with calibrated resistance and quantitative dissipation for biosensing measurements at higher harmonic frequencies". In: *Biosensors* 6.2 (2016), p. 23.

[R C19] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria, 2019. URL: https://www.R-project.org/.

[Wic16] Hadley Wickham. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York, 2016. ISBN: 978-3-319-24277-4. URL: https://ggplot2.tidyverse.org.