Study of Simultaneous Fluid and Mass Adsorption Model in the QCM-D Sensor for Characterization of Biomolecular Interactions

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Abstract: Increasing attention has been paid to application of the quartz crystal microbalance with dissipation (QCM-D) sensor for monitoring biomolecular interactions. This paper focuses on a practical application of protein-protein binding affinity measurement at low concentrations and minimal sample sizes (50-200 µl of 20-200 nM), which results in low signal measurement. A model simulating fluid flow, diffusion-convection, and mass adsorption within the QCM-D sensor was developed and studied using COMSOL Multiphysics. The simulated model shows that the onset kinetics of observed response curves is determined mainly by the mass transport rate of the mobile analyte. Effects of the feed concentration, flow rates, and binding rate constants on the sensor gram are also discussed. The study can be used to optimize the sensing conditions and guide determination of the affinity of biomolecular interaction.

Keywords: biosensor, QCM-D sensor, convection-diffusion, bioengineering

1. Introduction

The quartz crystal microbalance with dissipation (QCM-D) sensor has been widely used for its sensitivity and versatility. The QCM-D sensor allows for simultaneous measurement of frequency change (Δf) due to the adsorbed mass on its surface and energy dissipation change (ΔD) by periodically switching off the driving power over the crystal and recording the decay of the damped oscillation.

During past decade, the QCM-D sensor has become increasingly popular in the study of biomolecular interaction [1,2,3]. In the QCM-D sensor, the circular QCM crystal is mounted in the liquid cell chamber (inner diameter 11.1mm, height 0.64 mm). One of the reactants is immobilized on the sensor surface while the mobile analyte(s) flow continuously over it. A bound complex is formed on the surface of the crystal. A schematic QCM-D sensor and geometry of the sensor cell is shown in Figure 1.

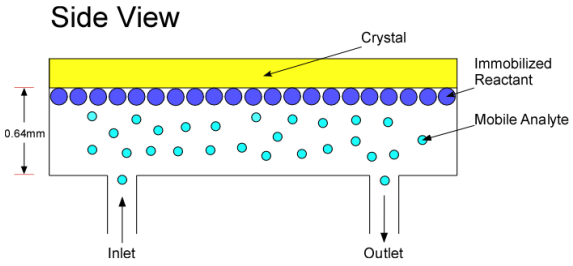

Figure 1. A schematic QCM-D sensor for biomolecular interaction monitoring (not to scale).

The purpose of this work is to elucidate phenomena underlying mass transfer, mass adsorption, signal measurement, and data analysis. The focus is protein-protein binding affinity measurements at low concentration and minimal sample size (50-200 µl of 20-200 nM) which generate only minute changes (0.1-2 Hz) in frequency. The fundamental understanding determines the applicability and the limit of the QCM-D sensor for estimation of affinity constants in biomolecular interactions at the specified conditions.

In this work, a coupled fluid and reaction-transport model in the sensing chamber is presented. Interaction between calmodulin (CaM, MW=16.7 kDa) and calcinuerin (CN, MW=28 kDa) was used as a model system.

2. Mathematical models

2.1. Governing equations

The QCM is placed in a thin circular liquid chamber that is designed to perform non-perturbed liquid exchange. The sample is drawn through the flow cell at a constant flow rate by a peristaltic pump downstream of the sensor. This Newtonian, incompressible fluid model can be described by the Navier-Stokes equation.

$$\rho\frac{\partial \boldsymbol{u}}{\partial t} + \rho(\boldsymbol{u}\cdot\nabla)\boldsymbol{u} = \nabla\cdot[-p\mathbf{I} + \mu(\nabla\boldsymbol{u} + \nabla\boldsymbol{u}^{T})]$$

with continuity equation $\nabla\cdot\boldsymbol{u} = 0$. Where **u** is the velocity (m/s), μ is the viscosity (kg/m·s). This fluid model was approximated as a steady-state flow.

The mobile analyte(s) flows over the chamber for a predetermined time as a continuous flow. The immobilized reactant and mobile analyte are referred to species A and B, respectively. For the mobile analyte B, the mass transfer equation applies as followings.

$$\frac{\partial c_B}{\partial t} + (\boldsymbol{u}\cdot\nabla)c_B = \mathrm{D}\nabla^2 c_B$$

Where c_B is the molar concentration of an analyte in the solution (mol/m^3) and D is the diffusion coefficient of the B molecules (m^2/s).

The initial condition sets the concentration of the bulk at the beginning of the process to zero.

c_B (at t=0) = 0

The reaction and mass adsorption of the biomolecules, occurs on the upper surface of QCM crystal gold electrode. For simplicity we assume pseudo first order reaction kinetics.

$$A + B \leftrightarrow A\cdot B$$

$$\frac{\partial c_{AB}}{\partial t} = k_f c_{B,s}(c_{A0} - c_{AB}) - k_r c_{AB}$$

Where c_{AB} is the molar concentration of a bound complex (mol/m^3), $c_{B,s}$ is the surface concentration of analyte concentration, and c_{A0} is the concentration of immobilized reactant A.

The initial condition sets the concentration of the bound complex to zero at the beginning of the process.

c_{AB}=0

2.2. Boundary conditions

No-slip boundary conditions are applied to all surfaces except at the inlet and outlet of the fluid chamber for the Navier Stokes model.

The boundary conditions for the material balance for the bulk are:

Inlet: $c_B = c_{B0} - c_{B0}H(t - V_s/Q)$

Where H is the Heaviside step function (for the COMSOL simulation, the smoothed Heaviside step function *flc1hs* was used), Vs is the volume of the analyte sample (m^3), and Q is the flow rate (m^3/s). The mobile analyte is applied for a predetermined time of $V_s/Q\,(s)$. The sample volume is set as 100 μl for the subsequent simulations unless otherwise stated.

Outlet: $\mathbf{n}\cdot(-D\nabla c_B + c_B\mathbf{u}) = \mathbf{n}\cdot c_B\mathbf{u}$

The bottom of the chamber is impenetrable, and the flux of the species B through the surface is zero, i.e.

$$\mathbf{n}\cdot(-D\nabla c_B + c_B\mathbf{u}) = 0$$

On the active sensor surface, the boundary condition couples the rate of reaction at the surface with the flux of species B.

$$\mathbf{n}\cdot(-D\nabla c_B + c_B\mathbf{u}) = k_f c_B(c_{A0} - c_{AB}) - k_r c_{AB}$$

3. Use of COMSOL Multiphysics

To calculate the bulk concentration of species B, combined multiphysics of the steady state fluid dynamics and convection-mass transfer modes was used. Since the model deals with a phenomenon in a 3D domain coupled to another phenomenon occurring only at the sensor surface 2D boundary, the PDE mode with weak form in the boundary was added. The weak form equation for the surface reaction is derived as follows:

$$0 = \int_{\Omega} v\,(k_f c_{B,s}(c_{A,sat} - c_{AB}) - k_r c_{AB} - \frac{\partial c_{AB}}{\partial t})dA$$

Where v is an arbitrary function on the domain Ω. The weak boundary equation for the upper

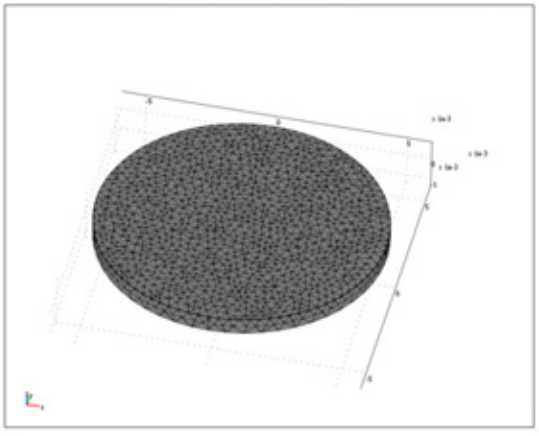

Figure 2. The mesh structure of the sensor cell

Table 1. Range of values used in the simulation

	low	medium (base case)	high
k_f ($M^{-1}s^{-1}$)	$2*10^4$	$2*10^5$	$2*10^6$
kr (s^{-1})	10^{-4}	10^{-3}	10^{-2}
c_{B0} (nmol/l)	50	100	200
c_{A0}(μmol/m^2)	0.23	2.3	23
Flowrate (μl/min)	75	150	300

surface was implemented in the COMSOL multiphysics using the *test* function: test(c_{AB})*(k_f*c_{B0}*(c_{A0}-c_{AB})-k_r*c_{AB} - c_{ABt}).

Since the reaction occurs only at the surface, the finer mesh should be defined near the surface (non equidistant). The reactive surface was divided with a finer mesh than the other side as shown in Figure 2. The mesh used in the model yielded approximately 85000 degrees of freedom. The transient convection-diffusion model was simulated for 240 seconds of time progress at an interval of 1 second.

Parameters considered in this simulation study are the forward and reverse rate constants (k_f and k_r, respectively), concentration of mobile analyte (c_{B0}), surface density of the immobilized molecules (c_{A0}), and flow rate (Q). The range of values for simulation study, shown in Table 1, are selected to represent values encountered in actual protein-protein binding experiments. The surface density of the immobilized molecules (c_{A0}) on the surface is calculated by Sauerbrey equation, corresponding to frequency change of 0.1, 1, and 10 Hz for low, medium, and high, respectively.

4. Results and Discussion

4.1. Fluid profile in the sensor cell

The continuous fluid profile in the QCM-D sensor cell was simulated. The flow is injected through an inlet port followed by continuous exit through the outlet at a controlled flow rate. Facing large area in the disk, the flow experiences significantly reduced the velocity as shown in Figure 3. The velocity of the majority of disk (at z=0.32 mm at the center of the disk height) is maintained at about 0.3-0.7 mm/s at the flow rate of 150 μl/min. The flow velocity at near upper surface where the reaction occurs

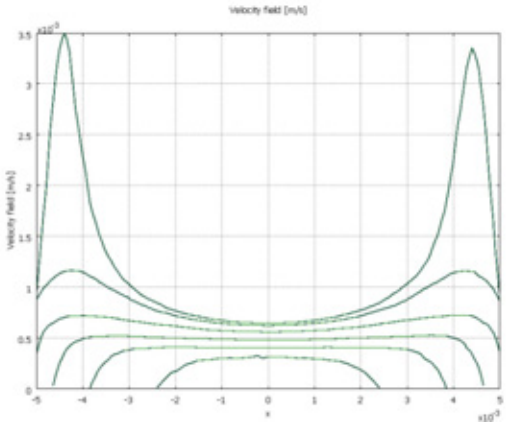

Figure 3. Velocity profile across the sensor chamber at different y slices. Flow rate=150 μl/min, y={0(center of the disk, the top line shown above),1,2,3,4,5(side of the crystal, the bottom line in the graph) }

(1 um away from the surface) reaches to a few um/s. The simulated result shows the non-perturbed laminar flow stream is established within the sensor chamber. The analysis of the velocity in the sensor chamber allows parametric study. The Peclet number, the ratio of convective mass transfer to molecular mass transfer ($Pe = LV/D$), ranges in the order of O(10^3). This indicates that the system is convective mass transport dominant. The Damköhler number (Da), the ratio between the reaction rate and the rate of convectional mass transport ($Da = k_f c_{A,sat}/V$) is calculated to be O(1). This indicates that the reaction occurrs in an intermediate regime where, although the mass-transfer rate is not strictly limiting, substantial concentration gradients can be present.

4.2. Concentration profiles

The mobile analyte B is applied to the sensor cell while the reaction occurs on the surface of the crystal where the reactant A is immobilized. Concentration of B gradually covers the surface and then diminishes as time progresses as shown in Figure 4. At the flow rate of 150 μl/min and 100 μl sample size, it takes 40 seconds for B to fill in and an additional 40 second to be washed away. The reaction occurs as the mobile analyte B is delivered to the upper surface. The corresponding surface concentration of the AB complex is shown in Figure 5. The concentration of bound complex AB keeps

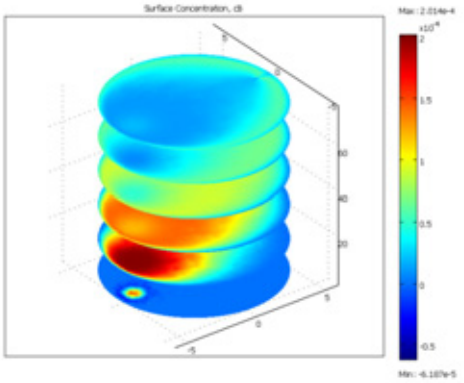

Figure 4. Transient concentration profile of the species B at the upper surface for time points of 1,

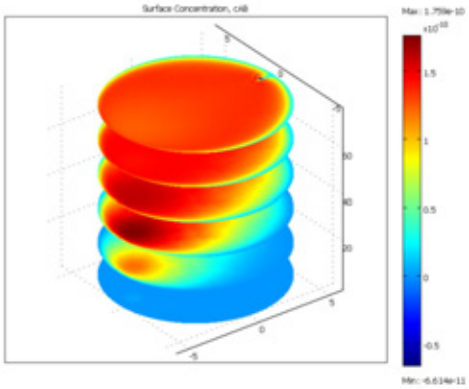

Figure 5. Transient concentration profile of the bound complex AB at the upper surface for time points of 1, 15, 30, 45, 60, 75 seconds.

increasing until the B molecules start effacing off when the mobile analyte B is completely washed off.

4.3. Simulated response curves

The QCM-D sensor measures the resonant frequency change during the binding of the dynamically coupled mass, including both bound analyte and trapped water. Although the simulation can demonstrate only the bound analyte mass, it nonetheless demonstrates the kinetics of the sensorgram during the mass adsorption. The response curve (ΔF) was simulated by integrating the boundary concentration of c_{AB} and dividing it by the disk area. The simulated response curves are shown in Figure 6 at different mobile analyte concentrations. This figure shows the QCM-D sensor signal is proportional to the feed concentration and thus can be developed to monitor/detect the unknown concentration of an analyte.

4.4. Effect of flow rates

To elucidate whether the kinetics obtained in the sensor gram can be used to estimate the binding kinetics of the biomolecular binding, the flow rate was changed. As shown in Figure 7, at a higher flow rate, faster onset kinetics in the sensor response were obtained. The result indicates that the mass transport of mobile analyte is mainly responsible for the obtained sensor response [4,5]. It is also notable that, at a slower flow rate, a significantly higher concentration of the bound complex AB is obtained.

The result demonstrates that the onset kinetics of the observed sensor gram cannot be used to determine the intrinsic binding kinetics. It also suggests that the low flow rate (75 µl/min) can result in higher sensor response signal so as to generate a higher signal to noise ratio. This provides more ideal sensing conditions for experiments that deal with the low concentrations and low sample volumes.

4.5. Effect of rate constants

The effect of binding kinetic constants on the sensor response was simulated by changing the forward and reverse rate constants individually.

When the forward rate constant (k_f) was changed, the onset kinetics of the simulated response remained unaltered although the total amount of the bound complex was increased at higher values (Figure 8). This result again

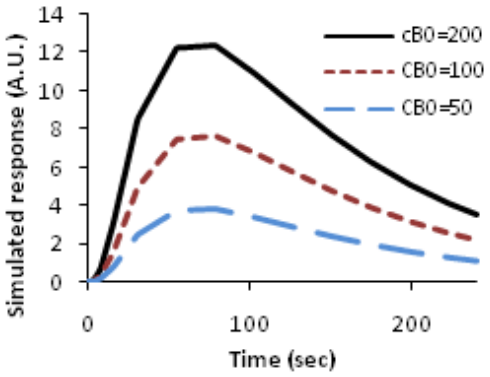

Figure 6. The simulated sensor response curves at various feed concentrations (in nM).

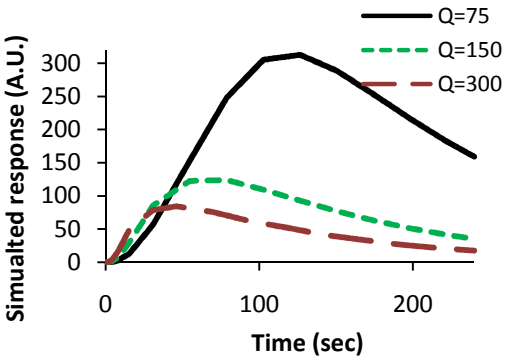

Figure 7. The simulated sensor response curves at various flow rates (in µl/min).

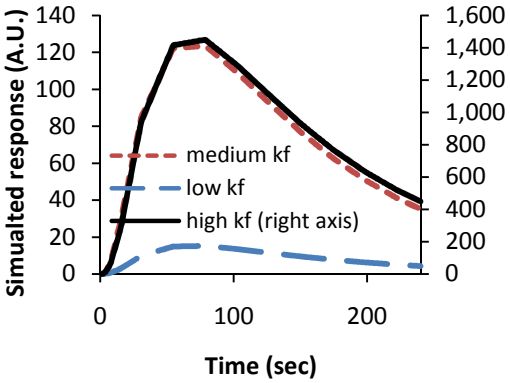

Figure 8. The simulated sensor response curves at various forward rate constants.

confirms that the onset kinetics of the observed sensor response is not affected by the intrinsic binding kinetics. Onset kinetic constants cannot be measured from the QCM-D sensor at these sensing conditions. Therefore, estimation of the affinity upon biomolecular interaction should be performed with its equilibrium (saturation) data.

When the reverse rate constant was changed, the kinetics of recovery rate were affected as shown in Figure 9. This indicates that reverse rate is sufficiently slow that it is the limiting factor.

5. Conclusions

A simultaneous fluid, mass transport, and reaction of biomolecules on the QCM-D sensor chamber was developed using COMSOL Multiphysics™. The fluid and analyte propagation at the active site of the QCM cell

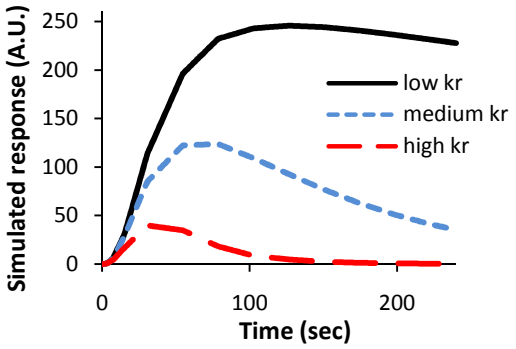

Figure 9. The simulated sensor response curves at various reverse rate constants.

was demonstrated. The simulated sensor response curve showed that the onset kinetics are mainly due to the mass transport rate of the mobile analyte while the dissociation kinetics are affected by the reverse rate constant. A flow rate of 75 µl/min showed significantly higher sensor response signal ideal for low signal measurement. This simulation renders understanding of underlying mechanisms in monitoring biomolecular interactions using the QCM-D sensor and can be used to optimize the sensing conditions.

6. References

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7. Acknowledgements

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