

# Using a QCM Biosensor to Measure Thermodynamic Properties of Protein-Protein Interactions

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## Introduction

In this study we show that quartz crystal microbalance technology (QCM) can be used to measure thermodynamic parameters such as the enthalpy and entropy contributions of molecular interactions. Accurate information of the thermodynamic parameters, for example if a protein-protein interaction is enthalpy or entropy driven, has already been proven useful in pharmaceutical research. Here we studied the interaction of a genetically engineered variant of protein A and the Fc-domain of two antibodies to verify the usefulness of the QCM technology in finding selection criteria for potential drug candidates. Data in this report were obtained by full kinetic analysis at a range of temperatures and final parameters were calculated using a Van't Hoff plot.

The thermodynamic properties of antibody-antigen interaction provides additional molecular information and thereby the possibility to select between antibodies that for example have similar kinetics. This QCM-based approach is also applicable to other types of molecular interactions.

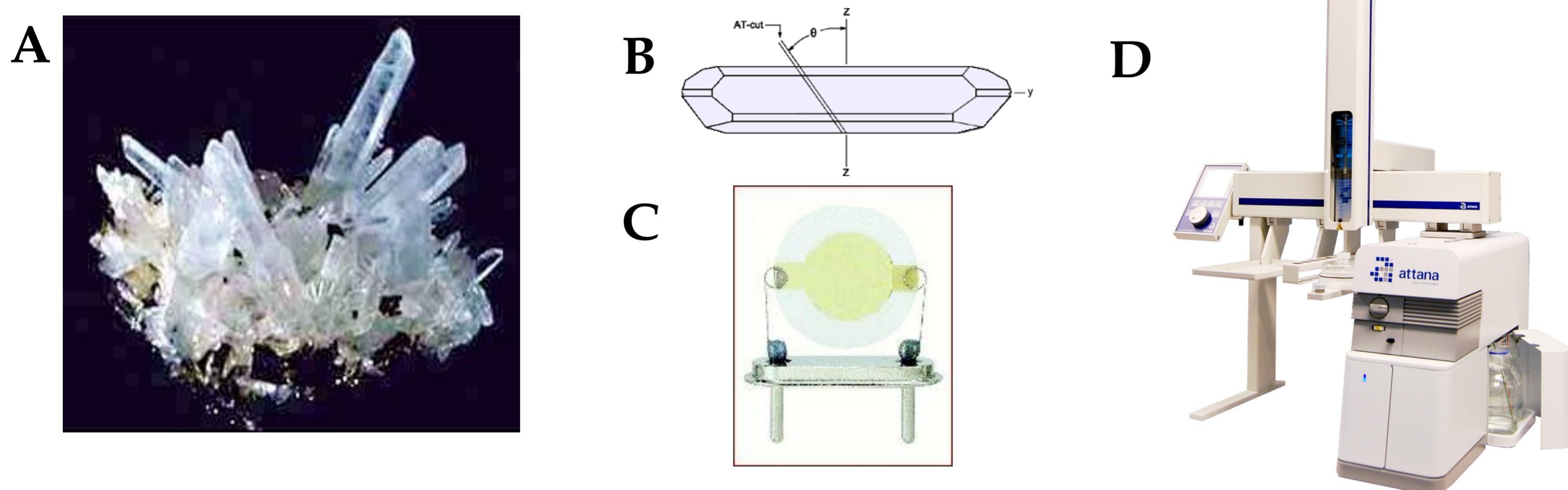


Figure 1. SiO<sub>2</sub> (A) is cut in a thin and very specific way (B). The gold electrodes subsequently coated on the crystals comprise the base for the surfaces used in the Attana sensor chips (C). The Attana A100 C-fast biosensor (D).

## Experimental procedures

Attana biosensors utilize the Quartz Crystal Microbalance (QCM) technique for real-time, label free measurements of molecular interactions. When molecules are added to, or removed from the surface, the change in the resonance frequency corresponds to the change in mass on the sensor surface. By immobilizing a target molecule to the sensor surface, and introducing an interacting molecule over the surface, the interaction can be studied in real-time. The real-time data can provide information on kinetics, affinity, active concentration and specificity for the interaction.

In this study we utilize the instrument for measuring equilibrium thermodynamic parameters such as  $\Delta H^\circ$  and  $\Delta S^\circ$  for the molecular interactions between the Fc-domains of two different antibodies and a genetically engineered biotin conjugated variant of protein A.

The biotin conjugated variant of protein A was immobilized onto a streptavidin-saturated biotin sensor chip. A rabbit anti-mouse antibody and a mouse monoclonal antibody were employed in this analysis. The two antibodies were separately flowed over the surface (25  $\mu\text{l}\cdot\text{min}^{-1}$ ) at 8, 16, 24 and 32°C at five concentrations for a global kinetic analysis (Fig. 2). The  $K_{\text{on}}$  and  $K_{\text{off}}$  were obtained for all temperatures and  $K_{\text{D}}$  calculated. The surface was regenerated between each kinetic cycle by exposure to 10 mM glycine-HCl pH 3.0 for 30 seconds. The surface showed no loss of activity during the experiment.

Thermodynamic measurements using van't Hoff analysis is an alternative method to isothermal titration calorimetry. Advantages of using biosensor data for this purpose are the relatively low consumption of samples and the simultaneous collection of kinetic data.

The equations  $\Delta G^\circ = RT \ln K_{\text{D}}$  and  $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$  can be merged to:  $RT \ln K_{\text{D}} = \Delta H^\circ - T\Delta S^\circ$ . This equation can be rewritten:  $\ln K_{\text{D}} = \Delta H^\circ/RT - \Delta S^\circ/R$  and be employed for linear plots where  $\Delta S^\circ$  can be obtained from the y-axis intercept ( $\Delta S^\circ/R$ ) and  $\Delta H^\circ$  from the slope ( $\Delta H^\circ/R$ ).

$R$  = the gas constant = 8.314 J · (K mol)<sup>-1</sup> and  $T$  = temperature in Kelvin

## Results

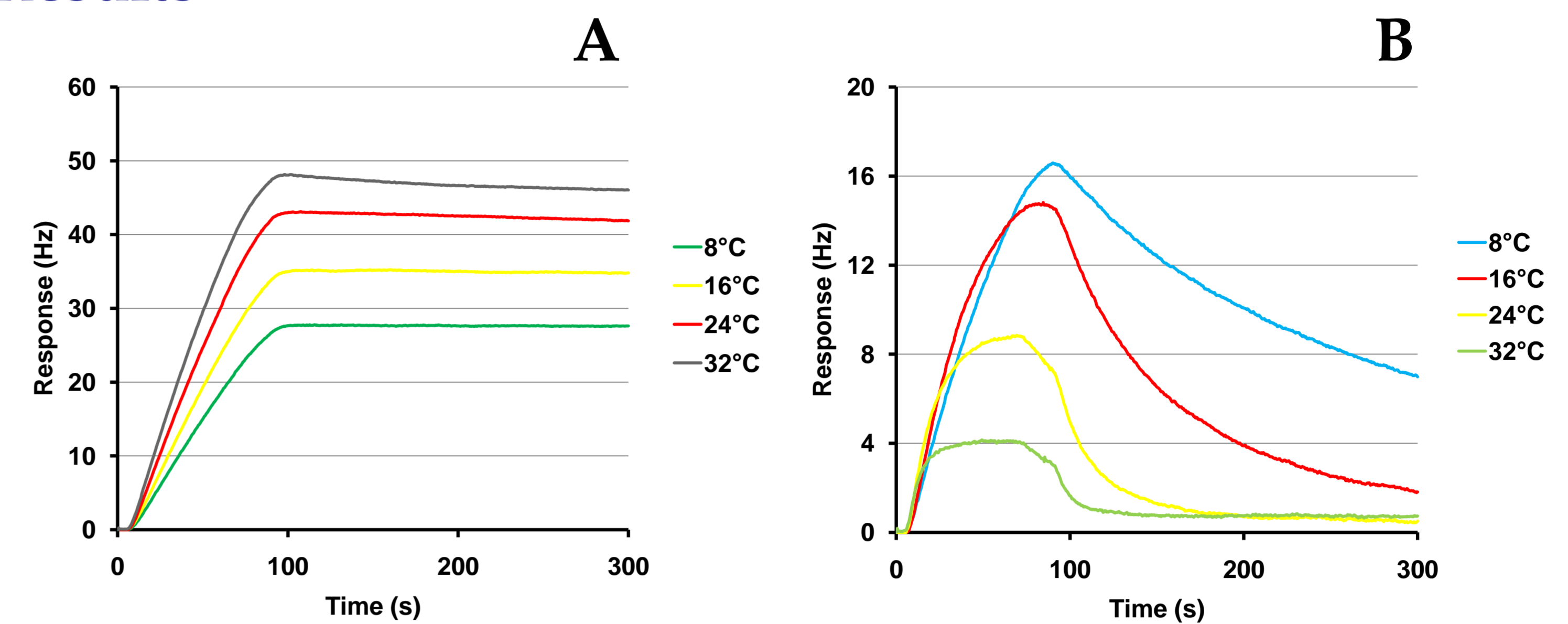


Figure 2. Sensograms showing (A) rabbit anti-mouse at 2.5  $\mu\text{g}\cdot\text{ml}^{-1}$  and (B) mouse monoclonal antibody at 5  $\mu\text{g}\cdot\text{ml}^{-1}$ . The Fc affinity for the genetically modified protein A surface is lower for the mouse antibody compared to the rabbit anti-mouse. The temperature dependence of the interactions is evident; and interestingly, the binding capacity of the mouse antibody is inversely proportional to temperature.

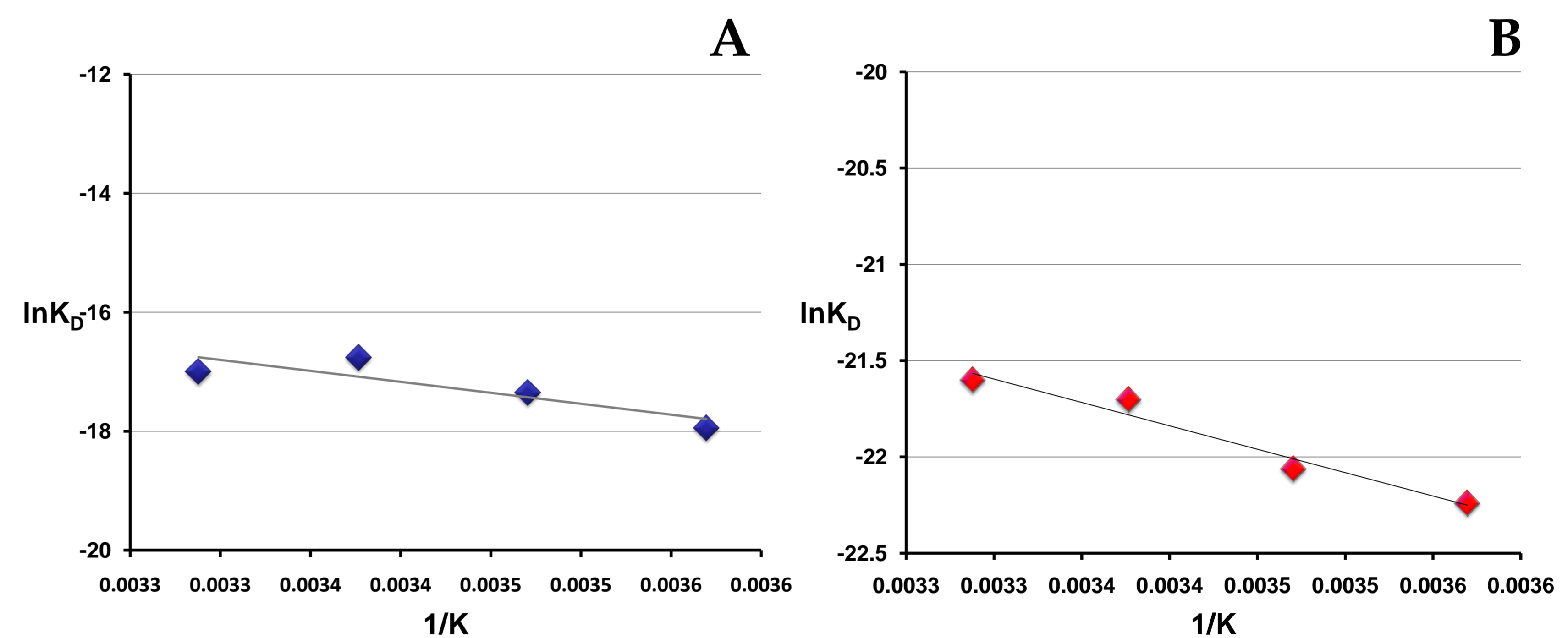


Figure 3. Van't Hoff plots of the genetically modified version of protein A and the Fc-domain of (A) rabbit anti-mouse and (B) mouse monoclonal antibody.

The following equilibrium thermodynamic data were calculated from these plots (Fig. 3).

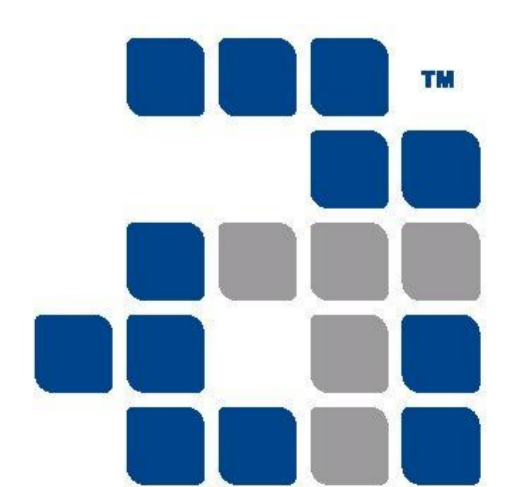
Mouse monoclonal antibody:  $\Delta H^\circ -444 \text{ J}\cdot\text{mol}^{-1}$ ,  $\Delta S^\circ 0.5 \text{ J}\cdot(\text{K}\cdot\text{mol})^{-1}$

Rabbit anti-mouseFc antibody:  $\Delta H^\circ -292 \text{ J}\cdot\text{mol}^{-1}$ ,  $\Delta S^\circ -1.6 \text{ J}\cdot(\text{K}\cdot\text{mol})^{-1}$

These preliminary data indicates that the interactions between the genetically modified protein A capturing surface and the Fc domain of these antibodies are dominantly enthalpy driven.

## Conclusions

- The QCM technology allow measurements of thermodynamic parameters such as enthalpy and entropy for non-covalent molecular interactions and provides an alternative method to isothermal titration calorimetry
- Thermodynamic properties can be useful additional parameters for selecting drug candidates
- This method is rapid and consumes small amount of samples



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