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Mini Review


# Recent Advances in using the Quartz Crystal Microbalance to Analyze the Binding of Drug Ligands to Serum Albumin Proteins

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There has been a dearth in understanding how interactions between drug molecules and macromolecules can inform rational drug design [1]. Understanding the thermodynamic, structural, and kinetic aspects of these interactions are key to progressing the development of novel drugs. Many of these studies utilize either bovine serum albumin (BSA) or human serum albumin (HSA) as their drug target [2-7]. BSA is a standard serum albumin protein that functions as a transporter of drugs [8]. HSA is a serum albumin protein found within human blood that functions as a transporter of various compounds, acts as an antioxidant, and plays important roles in drug delivery [2]. The use of these two serum albumin proteins in drug binding studies has led to a better understanding of the interaction between drug molecules and albumin [3,4]. Studies using HSA are particularly attractive to the pharmaceutical industry because of its ability to bind a myriad of drugs, giving insight into the drug's delivery and efficacy, while decreasing side effects of these drugs [5].

A recent analysis viewed drug research and development through the lenses of efficiency and efficacy, both of which relate to each other [9]. Target selection may be integral to overall drug research and development [9], but it only emphasizes efficiency in relation to examining a drug's ability to bind a specific target. The efficacy portion is not covered due to the inability to observe the functional response caused by the binding. However, understanding the efficiency of a drugs' binding profile can lead to further studies on its efficacy; Any information about the interaction between a drug and its target is beneficial to drug development. By altering the structure of drugs, there is the possibility of developing a drug that binds stronger, with a better orientation, and more specifically to its target.

The conventional way of quantifying binding involves obtaining kinetic profiles which are then used to determine the thermodynamic binding constant. The one-step binding of drug ($D$) to protein ($P$) follows a simple second-order process (Equation [1]), where $k_{on}$ and $k_{off}$ represent the rate constants for association and dissociation, respectively.

$$D + P \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} DP \qquad \text{[Equation 1]}$$

The solution to the rate law governing this process shows explicitly how the concentration of the complex ($[DP]$) varies with time as the drug is exposed to protein under a constant excess concentration of drug ($[D]_0$) (Equation [2]).

$$[DP](t) = [P]_o \frac{k_{on}[D]_o}{k_{on}[D]_o + k_{off}} \left(1 - e^{-(k_{on}[D]_o + k_{off})t}\right)$$

[Equation 2]

The exponential term in Equation [2] can be expressed as an observed rate constant ($k_{obs}$) (Equation [3]).

$$k_{obs} = k_{on}[D]_0 + k_{off} \qquad \text{[Equation 3]}$$

Thus, a nonlinear fit of data to Equation 2 provides a value for kobs. Determining various kobs as a function of $[D]_0$ then allows the simultaneous determination of $k_{on}$ and $k_{off}$. Finally, the ratio $k_{off}/k_{on}$ provides the thermodynamic binding constant ($K_D$).

One increasingly common method of studying drug binding is Quartz Crystal Microgravimetry with Dissipation Monitoring (QCM-D), which measures the piezoelectric response (frequency) of a quartz crystal as a result of coupling mass to the crystal surface (Figure 1). In short, this piezoelectric response can be used to provide real-time mass changes, specifically for the formation of $[DP]$ as a function of time while under a constant flow of $[D]_o$ [10].

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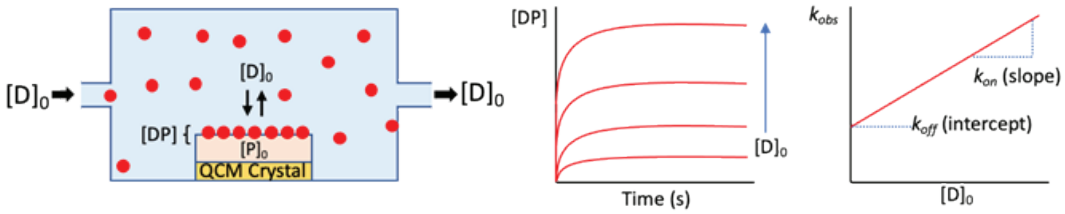


***Figure 1.*** *(left) Schematic of a typical QCM-D experiment. The drug D is passed over the piezoelectric surface (pre-coated with protein P). (middle) Binding profiles as a function of* $[D]_o$ *as described by Equation [2]. (right) The subsequent linear variation of kobs versus* $[D]_o$ *as described by Equation [3]*

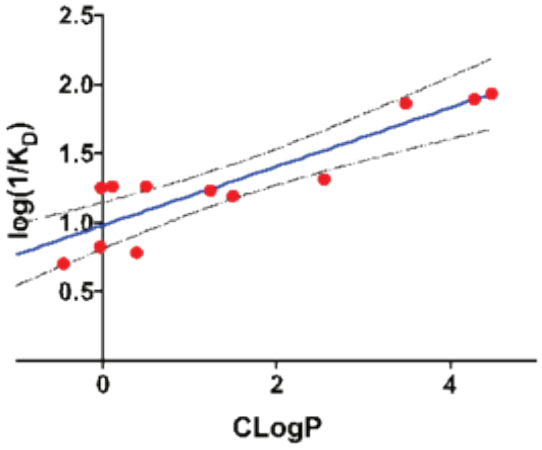


***Figure 2.*** *The relationship between logarithm reciprocal binding constants (*$log(1/K_D)$*) (as measured by QCM-D) and the hydrophobicities (*$C_{log}P$*) of various drugs.[3]*

The QCM-D's viability as a technique for studying drug binding is discussed through observing both the progress it has made within this field, as well as the limitation this method has. It should be noted that QCM-D is arguably simpler to operate (and certainly more cost effective) than more established methods such as Surface Plasmon Resonance (SPR) for these kinds of studies.

Many drug binding studies revolve around the use of HSA and BSA, used almost interchangeably given their many similarities, structure-wise [6,11,12] and reaction-wise [3,4,13,14]. Results using these two proteins have been practically identical, but minor differences have been revealed in the way they interact with specific drugs. In the case of commonalities, these proteins have similar binding profiles across a wide range of drugs [3,4], have disclosed quenching properties of certain antithyroid drugs [6], and more [13,14]. Minor differences have been found in certain binding profiles with some drugs [3,4,15], along with the fluorescence [16] of these proteins and other specific reaction differences [13,16,17], but the majority show similar reaction qualities.

Although HSA and BSA are very similar, using HSA as a drug target creates a closer translational link between findings within research and their applicability to humans [18-21]. Biological efficacy can be determined by examining the interactions between drugs and HSA. One important application of HSA is its ability to permeate the blood-brain barrier, a region the majority of drugs are not able to pass through. One limitation however, is that it is not able to hold cationic drugs within its binding region. A recent study [5] used this knowledge to attempt to manipulate the structure of HSA to be able to hold cationic drugs and successfully did so. This finding further strengthens the credibility that HSA has to progress the general field of medicine through drug delivery. This is one of many positive outcomes of the exploitation of HSA, along with improvement of the targeting of anticancer drugs and decreasing their side effects [5].

In recent years, the QCM-D has been readily used to further our understanding of the interactions between drugs and both serum albumins. Our laboratory has used the QCM-D to explore the degree of desolvation upon binding of drugs to BSA [7] and understand the effects of hydrophobicity on desolvation and drug affinity in both drug-BSA and drug-HSA interactions [3,4]. For example, Figure 2 shows a strong correlation between a drugs hydrophobicity (cLogP values) and the binding constant [7]. Outside of this, the general viability of the QCM-D for other methods has been tested, such as its ability to explore conformational changes upon drug-protein binding [22]. Additionally, the QCM-D's viability in use in series with other methods, such as SPR, dual polarization interferometry (DPI), and spectroscopic ellipsometry (SE), has been tested, and through all of these studies, the QCM-D has been shown to continue produce novel and significant findings [3,7,22-26].

There are some limitations regarding the use of the QCM-D and HSA within drug binding research: there is an inability to represent *in vivo* conditions within HSA studies [27], along with an inability to observe the functional response of the drug-HSA complex, and it is also unknown whether the structure and function of HSA are altered upon adsorption onto the QCM-D piezoelectric sensor surface [4]. *In vivo* conditions consist of 0.1 to 2.0 moles of fatty acids in contact with the binding sites of HSA and the bodily surroundings [27]. Future directions would address these limitations by attempting to mirror *in vivo* conditions within the QCM-D and supplementing the QCM-D with other methods [28] to observe the structure and function of the adsorbed HSA and the drug-HSA complex formed after binding.

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