# Determination of Active Concentration Using Calibration-free Methods

Surface Plasmon Resonance (SPR) is used to measure binding affinity and kinetics but it is also useful for determining the concentration of the analyte as it is a technique which is label-free, real-time, and mass-based allowing precise determination of active concentrations. The determination of active concentration in a sample is critical for accurate kinetics analysis and is crucial for pharmaceutical development, quality control, protein and biomolecule research.[1] Several of the existing approaches can measure sample concentration, but most require look-up tables or pre-calibrated response curves, and very few approaches like SPR are able to easily distinguish between active and inactive sample concentration.[2] SPR can determine active sample concentration by monitoring the magnitude of the binding signal and the mass change from the bound analyte.[3]

BI-SPR enables two simple calibration-free methods for measuring the active concentration of samples: **CD-KD (Concentration determination - affinity based)** and **CD-C (Concentration determination - reference concentration based)**. Depending upon the known criteria, one method may be more suitable than another. **CD-KD** calculates the unknown concentration when the sample affinity is known. **CD-C** calculates the unknown concentration by referencing a sample of known concentration.

CD-KD provides a quick, calibration-free method to determine the active concentration of analytes in the sample solution when the affinity is known. This method does not rely upon look-up tables or pre-calibrated standard curves, but rather is based upon the active binding response of the sample. In the example shown below, CD-KD method is used to determine the unknown concentration of anti-bovine serum albumin binding to the immobilized bovine serum albumin (BSA) by fitting the data in the injection series to a known affinity (KD). By using the known affinity for the aBSA-BSA interaction of 3.6 nM, a concentration of 100 nM was calculated as a best fit for the injection series. The calculation involving kinetic fitting yields the concentration which is listed in the plot legend which accurately matches the injected concentration value with an error of 2% in most cases. This affinity-based method is highly sensitive, able to detect even picomolar concentrations of analytes, and is particularly useful for studying mutant forms of proteins, where standards are often unavailable.

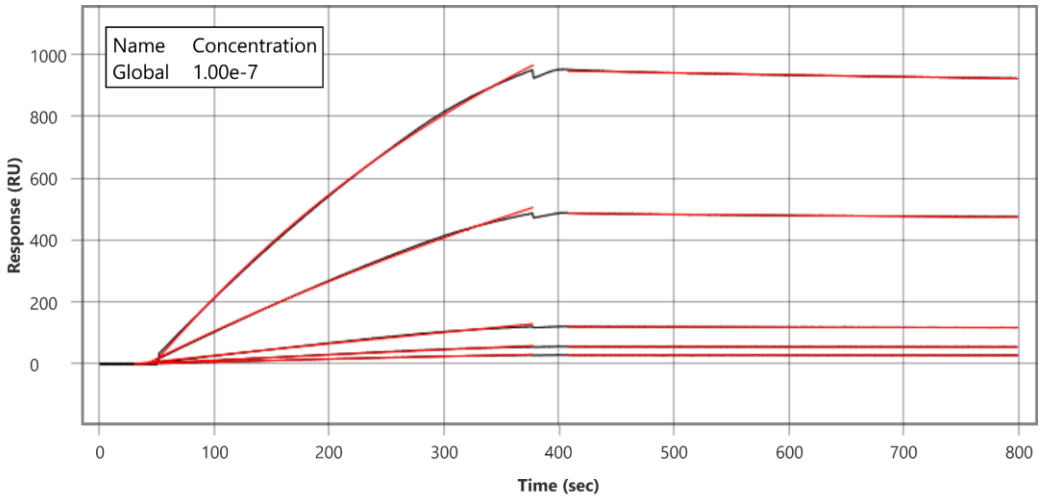


*Figure 1: Concentration determination of anti-BSA using CD-KD method obtained with BI 4500 system*

CD-C provides a quick, calibration-free method to determine the active concentration of analytes in the sample solution when a sample of known concentration can be referenced. In the example shown below, CD-C method is used to determine the unknown concentration of anti-bovine serum albumin binding to the immobilized BSA by referencing the binding response of a sample of known concentration. Here, foreknowledge of the sample's KD value is not required. By reference to the response from the known sample of concentration 200 nM, active concentration of the analytes were calculated as a best fit for the injection series listed in the plot legend. This referenced-based method is highly sensitive with an error of 1%, and is particularly useful for studying samples of unknown KD.

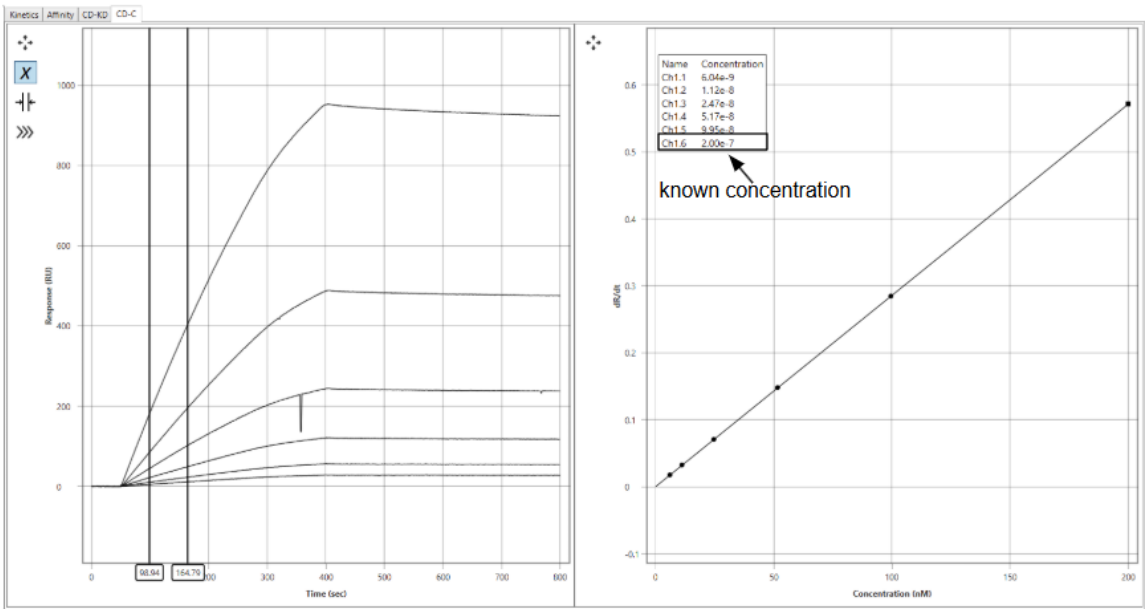


*Figure 2: Concentration determination of anti-BSA using CD-C method obtained with BI 4500 system.*

BI-SPR provides two simple methods for calibration-free determination of the active sample concentration. Simple and accurate determination of active concentration is critical for drug discovery, biomarker identification, purity evaluation, and high quality quantitate kinetics analysis.[4]

*References:*


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