# Biolayer Interferometry: Pho4 DBD and 17bp oligo ligand

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## Overview

* The purpose of this protocol is to describe the steps used to analyze the Octet data to estimate dissociation constants between protein-DNA binding.

## Reference

1. Data Analysis Octet, User Guide v11, stored on RDSS/Shared/Instrument/Octet.
2. Forte Bio Application Note 14, stored at the same place as above.

## Software

Data Analysis version 11.1.0.4, by Forte Bio

## Protocol:

### **Opening data for analysis**

See reference 1 above.

### Sensor selection

See reference 1 above. Used to exclude certain sensors for future analysis.

### Processing data

1. *Choose reference sensor*  
   The goal is to subtract the background using a reference sensor that moved in parallel with all other sensors, but was dipped into a solution of 1x KB instead of the analyte (protein) solution. In the data acquisition software during the setup stage, the user should have specified one of the wells to be a reference rather than sample. When you click the “Sensor Selection” radial button on the top left of the “Processing” tab, diagrams of both the sensor tray and the sample plate will show up on the right, and the well that was designated as reference will have a checkbox next to the row ID, e.g. A. Click that.
2. *Subtraction*  
   Click the checkbox and choose reference well.
3. *Align Y-axis*  
   The goal of this step is to align all traces to the same starting point to enable steady state curve fitting -- due to sensor-to-sensor variation and stochasticity in the baseline, loading and blocking steps, not all sensors will start from the same y-value at the start of the association phase. We typically choose **Baseline**, and set the time window such that it extends for about 3-5 seconds towards the end of the last baseline step, but doesn’t go right into the end. For example, if the last baseline step lasts for 40 seconds, we can set the time window to be **35-38**. The reason for this is because right at the transition between the baseline and the association, there could be jumps in the signal due to the time delay as the instrument physically moves the sensors from the buffer to the analyte wells.
4. *Interstep correction (optional)*  
   The goal of this step is to correct misalignment between two steps, e.g. baseline and association, due to system artifacts (perhaps due to the movement of the sensors).
   * For the most effective correction, the dissociation and the baseline steps of an assay must be performed in the same microplate well.
   * Interstep correction is *not* recommended for very fast kinetics, because some kinetic information may be lost.
   * For our purpose we use “**Align to dissociation**” to correct for jumps in the signal at the beginning of the dissociation phase. This is only important if we are going to do kinetics fitting rather than steady state fits.

### Kinetic analysis

There are two types of kinetic analyses:

* Curve fitting: determines *kon, koff* and *KD*by fitting the data to a specific binding model.
* Steady state: determines the dissociation constant *KD* from the calculated or measured equilibrium response.

#### Curve fitting

1. *Choose steps to analyze*
   * Association only: generates *kobs*
   * Dissociation only: generates *koff*
   * Association and Dissociation: generates *kon, koff, kobs* and *KD*
2. *Select model*
   * **1:1 Model**: one analyte in solution binding to one binding site on the surface
   * **2:1 Model**: one analyte in solution binding *two different binding sites* on the surface (heterogeneous ligands), with different kinetic parameters (*kon1, koff1*, *kon2, koff2*)
   * **1:2 Bivalent Analyte Model**:  
     one bivalent analyte in solution binding to a monomeric immobilized ligand
   * **Mass Transport Model**:  
     A two-step binding model that involves the analyze first diffusing to the surface of the biosensor and then the specific binding, where the first step is rate-limiting
   * Except for the high affinity sequences, the 1:1 model fits most of the data very well.
3. *Fitting local*  
   In this mode the software calculates kinetic constants for each curve.
   * **Full:** software assumes that dissociation will eventually reach the pre-association baseline value, and will force the curve fit to that point.
   * **Partial:** software doesn’t assume the above. Used to fit portions of curves in data sets with significant, or biphasic, dissociation. Note that this option tends to give higher *KD* values.
4. *Global Fit*  
   This gives the most accurate kinetic and affinity constants because it utilizes all the data
   * **By sensor:** do not apply to us
   * **By color:** group all the data that is the same “color” and applies a global fit to that group. Note that by default, the software will assign *different* colors to different sensors. To use the Global Fit option, first select all sensors in the table below the curves in the Analysis tab, right click and “Set colors”. Choose the same color.
   * **Rmax:** always choose the Rmax Unlinked by Sensor option, which allows for each sensor to have slightly different surface capacity, hence Rmax (aka Bmax).
5. *Window of interests*  
   We typically use the entire step time for the association, but only use the beginning portion (e.g. 60s) of the dissociation phase, which seemed to improve the fit judged by R2