# Biolayer Interferometry: Pho4 DBD and 17bp oligo ligand

## Reagents:

### 10 x kinetics buffer (10 mL)

| **Item** | **Catalog #** | **Stock conc.** | **Add (mL)** | **Final conc.** |
| --- | --- | --- | --- | --- |
| Tween-20 | Sigma or others | 10% | 0.02 | 0.02 |
| BSA | NEB B9000S | 20 mg/mL | 0.5 | 0.1% |
| 1xPBS, pH=7.4 | F/S #10010023 | 1x | To 10 mL | 1x |

For making 1x KB, dilute the 10x KB 1:10 with the same 1x PBS. Each experiment uses ~7 mL of 1x KB (1.6 mL for hydrating the sensor, 1.6 mL for buffer, 2 mL each for diluting the oligo and biocytin, and 1-2 mL for diluting the protein).

### Protein

Purified Pho4 DBD in 25 mM NaH2PO4, 0.5 M NaCl and 0.5 M THP buffer, stored at 4C, good for about a month. The concentration should be > 1.5 mg/mL and can be aliquoted in 300-500 μl

### Oligo

10 uM (based on the biotinylated strand) annealed. Link to oligo sequences and IDs [here](https://docs.google.com/spreadsheets/u/0/d/1ayFLmaEQWC5KL2AWXR1dca7oWqA9YfdvZnHMiRrGySU/edit) under “Ligand Information”. Protocol for annealing below.

### Biocytin

1 mg/mL stock provided by the P&CF

## Protocol

### Annealing oligo

Do this before the BLi experiment.

* Upon receiving the synthesized biotinylated and regular (complementary) oligos, resuspend the oligo in the IDT duplex buffer to 200 uM final concentration.
* Turn on the Eppendorf ThermoMixer and set the temperature to 99C
* Mix the following ingredients in a 1.7 mL microfuge tube

| **What** | **How much** |
| --- | --- |
| Biotinylated oligo | 5 uL |
| Complement oligo | 10 uL |
| IDT duplex buffer | 85 uL |

* Place the tube in the ThermoMixer and incubate for 5 minutes.
* Set the temperature to 25C, and leave the tube on the mixer until the temperature reaches 25C. Remove the tube(s) and put on ice for 5 min, then store at -20C.

### Hydrate the sensors

It is *important* to have the sensors hydrated for *1 hour or longer*. Therefore, as soon as you arrive at the core, prepare a tray with a black plate at the bottom and dispense 200 uL 1xKB in each well. Use the special multichannel pipette to transfer new biosensors to the plate. Note: one option is to hydrate the sensors the night before the experiment. Once hydrated, the sensors should stay hydrated and are good for at least a week.

### Prepare the protein

1. Chill the refrigerated centrifuge by closing the lid. To speed up the chilling, spin at 4000 rpm and it takes ~8 minutes for the temperature to go from RT to 4C.
2. (Optional) Start the DynaPro NanoStar if doing DLS measurements.
3. Get an ice-bucket filled with ice ready.
4. When the centrifuge is chilled, spin down the protein aliquot at 13,000 rpm for 10 min.
5. Measure the concentration of the protein using nanodrop, with the S75 buffer as blank.
   1. Use 2 μl of protein or blank for each measurement.
   2. Make 3 measurements for each sample and take the average.
   3. Pay attention to getting the extinction factor and MW correctly typed in. Note that the A280 10mm is what the instrument actually measures, while the mg/mL is calculated from that value using the extinction factor and MW.
   4. If the concentration is >20% lower than the last time it was measured, or if the three independent measurements vary a lot, it is likely that the protein quality has gone down. Consider doing an DLS to examine the level of aggregation in the sample.
6. If doing DLS, follow the [DLS protocol](https://docs.google.com/document/u/0/d/1v2xH5VOL_tmzeCO5WFiBHpjrcAYHWQKiE9QWT2owEb8/edit). Good protein should be within range 1 (1-10 nM) and have an average %PD (polydispersity) at around 5%. However, even with %PD as high as 10%, and with some slight sign of aggregation, e.g. 99.5% by Intensity in Range 1, the sample is likely still good for BLi.

### Plan the experiment

1. Open the [master spreadsheet](https://docs.google.com/spreadsheets/u/0/d/1ayFLmaEQWC5KL2AWXR1dca7oWqA9YfdvZnHMiRrGySU/edit) for BLi and add an entry in the BLi info sheet.
2. Open one of the template spreadsheets (for [ScPho4](https://docs.google.com/spreadsheets/u/0/d/15zIUM0KWDPyda9uqpADV2S-FgkEJfgJSYGqbfHXJoTM/edit) or [CgPho4](https://docs.google.com/spreadsheets/u/0/d/14PEEyiqMr1BrhxWasQOloLYX-HHDjmcgf44-iMh8W6o/edit)). Duplicate one of the existing sheets and change the oligo information, title, concentration of the protein sample and the titration series.
   1. The titration series vary by the oligo’s Kd. A general rule of thumb is to cover between 10x and 1/10 of the KD, with 7 x 2-3 fold dilutions. For example, if the KD (based on previous measurements) is ~ 10 nM, the titration series ideally covers 100 nM to 1 nM.
   2. For high affinity sequences, the protein stock will be too concentrated. Make a 1:10 or 1:50 dilution first, and use that as your “stock”.

### Dilute reagents

1. Thaw the annealed oligo, biocytin stock.
2. Make 2 mL of 35 nM oligo by adding 7 μl of the 10 uM stock to 1993 μl of 1xKB.
3. Make 2 mL of 1 ug/mL biocytin by adding 2 μl of the 1mg/mL stock to 2 mL of 1xKB  
   *It’s easier to prepare 5 mL of 1ug/mL biocytin in a 5mL tube, which would be good for 3 experiments.*
4. Use the spreadsheet above to make the dilution for the protein.

### Set up the experiment

1. Start the data acquisition software for the instrument, which will also warm it up.
2. Aliquot the reagents into four columns within a black 96 well plate following the template you set up above, in the following format

|  | 1 | 2 | 3 | 4 |
| --- | --- | --- | --- | --- |
| A | 1xKB | 35nM Oligo | 1ug/ml Biocytin | 1xKB Reference |
| B | 1xKB | 35nM Oligo | 1ug/ml Biocytin | [Protein]7 |
| C | 1xKB | 35nM Oligo | 1ug/ml Biocytin | [Protein]6 |
| D | 1xKB | 35nM Oligo | 1ug/ml Biocytin | [Protein]5 |
| E | 1xKB | 35nM Oligo | 1ug/ml Biocytin | [Protein]4 |
| F | 1xKB | 35nM Oligo | 1ug/ml Biocytin | [Protein]3 |
| G | 1xKB | 35nM Oligo | 1ug/ml Biocytin | [Protein]2 |
| H | 1xKB | 35nM Oligo | 1ug/ml Biocytin | [Protein]1 |

1. Place the tray with rehydrated biosensors along with the experimental 96 well plate into the instrument.
2. In the data acquisition software, open a new kinetics experiment with the experiment wizard or open an existing method.  
   *It’s easiest to find a previous experiment with a similar setting and use its method as a template. Simply navigate to that experiment’s folder and you will find a method file.*
3. There are five tabs. Tab 1 is for entering the reagent plate. We need four columns per experiment, namely buffer, load (oligo), load (biocytin, for blocking) and sample (we use top well for reference, i.e., buffer only); Tab 2 is for setting up the program (moving of the sensors); Tab 3 is for specifying the sensor locations in the sensor plate (please record the lot number). Tab 4 is to review the experiment and Tab 5 is to set the path and filename to save the data. When everything is entered, hit GO.

### Data analysis

[See this protocol](https://docs.google.com/document/u/0/d/1z-dBELVe8qtDPbY7EUSNsu1FsokRWpgNVChHTpqlwNY/edit)