Suggestions for Successful Binding Experiments

These suggestions are based on personal experiences with the instrument and methods obtained or derived from Dave Myszka's group. They pertain only to the operation and maintenance of the machine. Experimental design is up to you. Example methods files are in the Bjorkman directory on the PC. These are actual methods files I've used for various data collections (one analyte, multiple analytes, multiple analytes at multiple temperatures, chip activation procedures, etc.) You can save these out to your own directory and modify them for your own experiment to help you get going faster.

Starting up

You should find the machine with either a maintenance chip or the chip from a previous run still docked in the machine. The next few steps will destroy any protein on the sensor surface so make sure the previous individual doesn't still want their chip. If it's the maintenance chip don't worry about it.

1) Make sure the machine is primed in water. Begin the desorb procedure (under working tools). Follow the instructions. One of the desorb solutions is kept on the low shelf next to the Biacore, the other can be found on the second shelf on the door of the fridge under the Biacore computer. Time estimate=25 minutes. While desorb is running, make a note in the Biacore log on top of the instrument that you have performed this task. The Biacore software often complains that certain maintenance procedures have not been completed for weeks to months. This is a bug in the software and is often a blatant lie. Use the log book, not the software. Other cleaning procedures can be performed after desorb such as sanitize. Only use sanitize if it hasn't been performed for at least a month or if you have a specific reason to. Let's try to minimize the number of times we expose the system to bleach.

2) Undock the new chip and dock your chip. You must reprime the machine after each chip change (frankly it doesn't give you any choice). This is a good time to switch into your coupling buffer. **Note: If you are going to activate and using amine coupling, use a buffer that does not contain azide. Azide is weakly nucleophilic and somewhat reduces coupling efficiency. You will still be able to bind protein, but may lose coupling activity faster and not be able to achieve higher densities if desired.**

Chip preconditioning

I don't know why this works, but if you need maximum sensitivity and lowest noise (for example if you're using low coupling densities, and frankly, who doesn't want the cleanest data they can get) then I recommend using the chip preconditioning procedure. There are several methods used by various groups but the one I recommend is posted on the Biacore on a yellow post-it. It involves injections of four different solutions (sodium

hydroxide, hydrochloric acid, SDS, and phosphoric acid). You will do TWO injections of each solution of 20 µl at 100 µl/min flow rate. For your convenience a method file exists that will run that for you. It's in the Bjorkman users directory where all of our lab's data is stored and is called chip_preclean.blm. It will require you to save to a result file which honestly you will never need. You can overwrite the results file that is there called doesntmatter.blr or save it to your own directory. You'll never need this file. You will need to put 70 µl of each solution into the sample vials.

I have tested the machine with and without chip preconditioning. The result is a chip with smaller changes in response due to injections and a flatter baseline. This will not be very significant for datatsets using higher coupling densities but is recommend for lower densities and is REQUIRED for small molecule work.

Chip coupling

This section applies only if you are coupling a chip with your own proteins or antibodies. If you are reusing a chip or are using a pre-coupled chip (SA chip for example) you can skip this.

1) Coupling regents are aliquoted in sample vials in the –20 freezer facing into bay 351 B. Grab one tube of EDC and one tube of NHS. They contain enough solution to use in an activation procedure requiring 50µl of solution from each tube. I recommend using the mix_act_4cell.blm method file and takes approximately 20 minutes to run.

2) For speed I recommend activating all four flow cells at the same time and then coupling each one. Some people prefer to activate, couple, and block EACH flow cell, but since it takes 20 minutes to activate you will spend nearly 1.5 hours just waiting for the coupling and another hour to block all four one at a time. If you activate ALL flow cells simultaneously, you can then begin a sensorgram flowing over all four flowcells. Once that has begun you then change the flow path to whichever flow cell you wish to couple, and then move onto the next one. It is important to remember to change the flow cell each time you want to couple a new protein. The advantage to coupling in this manner is that it a) saves time because it takes a couple minutes to stop a sensorgram and another minute to start one so this way you only do that once and b) all of your coupling data is kept in one file for easy access. I recommend naming your coupling file such that the contents of each flow cell are listed to speed analysis of your data later.

3) If you are coupling the same thing to all four flow cells (e.g. the anti-his antibodyfor a capture experiment) then you want to flow your coupling mix over all cells simultaneously. Not all flow cells are created equal and after the first injection you will probably have your desired coupling density on one cell but have to use subsequent injections on the other three to equalize the response.

4) Blocking: There is a 1 L container of 1M ethanolamine pH=8.0 on top of the Biacore. I usually block with three 50ul injections of this solution at a flow rate of 10 µl/min. If you are rushed you can get away with one or two.

Experimental suggestions

Once you have coupled a chip you're likely ready to run an experiment. This next section assumes you've already worked out concentration ranges for your analyte(s) and regeneration conditions. To get optimal data for your run I suggest a couple of things be included in your method files:

1) Buffer blanks: A procedure called "double-referencing" has become quite popular recently and is a really good idea. To do this effectively you include the injection of several buffer samples throughout the run of your analytes. I usually do about 1/3 as many buffer blanks as I have analyte samples and space them evenly throughout the run. It is important to have one or two at the very beginning. Sometimes, especially after cleaning, the machine 'settles out' a little and the most drift will be seen with the first blank. After that it's fine. These blanks can be subtracted from the datasets once the contributions from the reference flow cell are subtracted. Any systematic drift over the course of the injection, the occasional drifting baseline, or in the best case will simply lower noise levels (helps with low coupling densities, REQUIRED FOR SMALL MOLECULE WORK).

2) Dipneedle: This command followed by a rack position identifier predips the autosampler needle into a vial containing your running buffer. It aspirates 10µl of the running buffer to rinse the needle and prevent sample to sample carryover. It also seems to smooth the transition between the running buffer and sample during the injection procedure. This command goes just before your injection command.

3) Kinject: This injection procedure provides for the smallest dispersion effect between running buffer and sample contact on the chip. This minimizes injection artifacts which can sometimes obscure the important first few seconds of the association phase. This injection method requires an additional 40µl of sample beyond the actual amount injected.

4) Flow rate: For kinetics you should use higher flow rates such as 50ul/min or more even if you don't have mass-transport effects in your system. This reduces injection spikes and the injections are more aligned in time (remember the flow cells are connected in serial so at low flow rates there is a significant time lag between flow cells and reference subtraction doesn't work near the beginning or end of injections. This is of course ok if you're doing equilibrium work.

5) Normalize: There are variations in the reflectance characteristics of the gold-dextran surface both between chips and between the same chip at different temperatures. You should always normalize when changing chips or temperature. There is a solution of 40% glycerol in the Biacore fridge. Aliquot 200μl of this solution into a vial and run the normalize procedure in working tools. The program asks for 500μl of solution but that's just trying to get us to waste it. It won't use anywhere near that amount. Normalizing takes about 7 minutes. For that reason I build it into my methods files so I don't have to wait around for it, and the rack position for the normalization solution can be specified instead of using the default. I prefer to normalize after the chip is cleaned, coupled, and blocked so it is as close to the experimental conditions as possible. Obviously this is a bad idea if your protein isn't happy in 40 % glycerol. This is rarely true but can happen. This could be especially bad if you are working with a metabolic protein or one that binds glycerol.

6) "The 5 keys to success in Biacore: PRIME PRIME PRIME PRIME PRIME," says Dave Myszka. The prime procedure flushes buffer through the system and is supposed to exchange it. Apparently it's not perfect. I recommend priming the system three times in your binding buffer before starting any sample injections. Again, this is something that can be built into your methods file so you don't have to stand there for a 1/2 hour waiting around for it.

Data Analysis

Two suggestions: Scrubber and Clamp. Both are loaded on the PC in the office. Scrubber is incredibly straight forward to use. . . just follow the tabs. Clamp is more complicated and someone should probably give you a quick intro. BIAevaluation is competent for fitting simple models but data preprocessing is unnecessarily difficult.

For more information about proper Biacore experimental design and information on using Clamp also see http://www.cores.utah.edu/interaction/

Good luck,
Tony