Automated processing of large PA channel peptide binding IV datasets
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Manual download: http://mcb.berkeley.edu/labs/krantz/scripts/perl/Ivbind/ivbind-manual.pdf

Processing a Single Directory

1. Collect IV peptide binding datasets using `pCLAMP`. The data is automatically saved as a serially time-date stamped file with the binary format called ABF. Save each ABF file also as an ATF text file for PA channels alone and for each peptide concentration tested.

2. Use the "Open Multiple IV *.atf file" button to check the data atf files in `ORIGIN6.1`. This is the button indicated (^) below on the toolbar.

^

You could open one file at a time with the single-ASCII button next to "Open Multiple IV *.atf file" if you want:

^

3. Graph the results by plotting the Voltage versus the Current columns (x vs. y). If the data for each concentration of peptide are reproducible, then proceed through the following analysis using `PERL` software: two modules, called `Hoh.pm` and `Ivbind.pm`, and one script called, `ivbind-script.pl`.

These software are downloaded from the web at:
http://mcb.berkeley.edu/labs/krantz/scripts/perl/Ivbind.zip

First set-up the experiment file in notepad or any text editor. The file will be called `exp.txt`. It will be saved in the same directory with your ATF files and the `PERL` software modules and script. The file will have the following format:

```
FILE,CONC
14303004.atf,0
14303005.atf,0
14303007.atf,10
14303008.atf,10
14303009.atf,30
14303010.atf,30
14303011.atf,100
14303012.atf,100
14303013.atf,300
14303014.atf,300
14303015.atf,1000
14303016.atf,1000
```

Note that the first line states the name of each of the two columns: 'FILE', and 'CONC'. These names are the ATF filename and the peptide concentration in nanomolar.

4. Now execute the `PERL` script, `ivbind-script.pl`.

```
perl ivbind-script.pl exp.txt
```

5. Now all the `exp.txt`, `BIN.txt`, `FIT.txt`, and the `MASTER.txt` files created by the `PERL` script can be mass imported into a new `ORIGIN6.1` project using the multi-ASCII import menu option under the File menu. It is important to create a new project so that there can be a new MASTER worksheet.

6. Now run the following ORIGIN script to fix column naming artifacts in the BIN and FIT and MASTER worksheets and to add error bar column types and so on. Note these Loops use the indexes 0 to 20. These are the number of voltage bins from -80 to +20 mV. If those settings change then adjust accordingly, i.e., if you have more than 21 voltage bins. The name of the script is:

```
ivbind-wks-format.ogs
```

The script should be placed in the Origin6.1 folder. Execute the script by this command in the script window:

```
run.file(ivbind-wks-format.ogs);
```

Alternatively, here is the text of the script file, which can be run from the script window:

```
#LOOP to format BINS worksheets after import from PERL
LOOP(ii,0,20) {
%K = BIN$(ii);
%K!wks.col3.name$="I";
%K!wks.col4.type=3;
%K!wks.col6.type=3;
%K!wks.col8.type=3;
%K!wks.col10.type=3;
  LOOP(jj,1,11) { %K!wks.col$(jj).width=12; };
win -i %K;
};

#LOOP to format FIT worksheets after import from PERL
LOOP(ii,0,20) {
%K = FIT$(ii);
%K!wks.col3.type=3;
  LOOP(jj,1,5) { %K!wks.col$(jj).width=10; };
win -i %K;
};

#Format of the MASTER worksheet after import from PERL
%K=MASTER;
%K!wks.col4.type=3;
%K!wks.col6.type=3;
%K!wks.col9.name$="DURBINWATSON";
LOOP(jj,1,9) { %K!wks.col$(jj).width=12; };
```

7. For graphing, this script can be run. But first be sure to add the following two ORIGIN graphing template files into the ORIGIN6.1 program directory:

```
ORIGINIV.OTP
ORIGINRESIDUE.OTP
```

Now the FIT worksheets can be plotted in ORIGIN using the following ORIGIN script.

```
ivbind-plot.ogs
```

The script should be placed in the Origin6.1 folder. Execute the script by this command in the script window:

```
run.file(ivbind-plot.ogs);
```

This is the contents of the file ivbind-plot.ogs, which can be run from the script window. As you can see the file is long so running the script can be easier than cutting and pasting.

```
#Script to plot the FIT worksheets in GRAPH windows
if("%P"=="") %P=PA WT;
if("%Q"=="") %Q=L-Trp;
if(ph==0/0) ph=5.6;
getn (PA) %%P
(PEPTIDE) %%Q
(pH) ph
(Parameters);

LOOP(ii,0,20) {
 %K=FIT$(ii);
#get info from master
jj = ii + 1;
```

```
volt=MASTER_V[jj];
volt=round(volt,1);
slope=MASTER_SLOPE[jj];
slope=round(slope,2);
slopeerr=MASTER_SLOPEERR[jj];
slopeerr=round(slopeerr,2);
intval=MASTER_INT[jj];
intval=round(intval,2);
interr=MASTER_INTERR[jj];
interr=round(interr,2);
rsquared=MASTER_RSQUARED[jj];
%T=MASTER_FILE[jj]$;
 win -t plot ORIGINRESIDUE;
 layer -s 1;
 layer -i %K_LOGQ;
 layer -i %K_LOGQERR;
 layer -i %K_FIT;
 layer -g;
 layer -at;
 xb.text$="Log %Q /nM";
 yl.text$="Log fo/(1-fo)";
 title.text$=Hill Plot Bin$(ii) at $(volt) mV for %P and %Q at pH $(ph);
parms.text$=Linear Fit of %T
R^2   $(rsquared)
slope $(slope) ± $(slopeerr)
intercept   $(intval) ± $(interr);
legend;
LEGEND.X=2.753654;
LEGEND.Y=1.308479;
layer -s 2;
layer -i %K_RESIDUE;
layer -at;
win -r %H GRAPH$(ii);
win -i %H;
};

#Graph Hill Coefficients
 win -t plot ORIGINIV;
 layer -s 1;
 layer -i MASTER_SLOPE;
 layer -i MASTER_SLOPEERR;
 set MASTER_SLOPE -k 2;
 set MASTER_SLOPE -kf 1;
 set MASTER_SLOPE -l 0;
 xb.text$="Voltage (mV)";
 yl.text$="Hill Coefficient";
 title.text$=Hill coefficients vs Voltage for %P and %Q at pH $(ph);
 legend;
 layer.X.from=30;
 layer.X.to=-90;
 layer.X.inc=20;
 layer.Y.from=-0.5;
 layer.Y.to=-2.5;
 layer.Y.inc=-0.5;
 layer.X.label.divideBy = -1;
 layer.Y.label.divideBy = -1;
 layer -a;

#Graph -Log Kdapp versus Voltage
 win -t plot ORIGINIV;
 layer -s 1;
 layer -i MASTER_INT;
 layer -i MASTER_INTERR;
 set MASTER_INT -k 2;
 set MASTER_INT -kf 1;
 set MASTER_INT -l 0;
 xb.text$=Voltage (mV);
```

```
 yl.text$=-Log K\-(D)app \/nM;
 title.text$=Log KD-app versus Voltage for %P and %Q at pH $(ph);
 legend;
 layer.X.from=30;
 layer.X.to=-90;
 layer.X.inc=20;
 layer.X.label.divideBy = -1;
 layer -a;

#Graph R-SQUARED versus Voltage
 win -t plot ORIGINIV;
 layer -s 1;
 layer -i MASTER_RSQUARED;
 set MASTER_RSQUARED -k 2;
 set MASTER_RSQUARED -kf 1;
 set MASTER_RSQUARED -l 0;
 xb.text$=Voltage (mV);
 yl.text$=R-Squared;
 title.text$=R-Squared versus Voltage for %P and %Q at pH $(ph);
 legend;
 layer.X.from=30;
 layer.X.to=-90;
 layer.X.inc=20;
 layer.X.label.divideBy = -1;
 layer -a;
```

8. Now create a Layout in ORIGIN to insert the Hill, Log Kdapp and R-squared versus voltage graphs (Graph21, 22, and 23) for printing. The New Layout button is indicated (^):

To make add Graphs 21, 22, and 23 to the Layout, you right-click on the Layout1 window and add the respective graphs. Rescale them as needed. Print out the layout, and paste a copy into your notebook.

9. SAVE your ORIGIN project in your data folder with your original ATF files!

ADDITIONAL SCRIPTS & METHODS

A. Merging Bin Datasets from Different Directories

Requires Ivbind.pm (version 0.2) and ivbind-merge-script.pl script.

1. You may need to merge bin datasets from several directories. You will make a new directory of the current date and copy the scripts to that directory. For example, in Cygwin:

```
mkdir 04292014/
cp /cygdrive/e/Ivbind/*.p* /cygdrive/e/Eric/04292014/.
```

2. Within this directory, you then call the ivbind-merge-script.pl script and the list of directories you will be merging. You use the leading '..' on the directories to tell the program to go back one directory to find your data directories:

```
perl ivbind-merge-script.pl ../04112014/ ../04212014/
```

The script looks like this:

```
use Ivbind;
my $iv = Ivbind -> new();
$iv -> delimiter(',');
$iv -> merge_bin_dirs( [ @ARGV ] ) -> save_bins -> hill_analysis;
```

Within your new directory, you will have the new merged bin files and fit files and a master file. These

can be imported into your ORIGIN project.

3. Follow steps 5-9 in **Processing a Single Directory** above to load your data into your ORIGIN project.

B. Removing Concentrations from Bin Datasets in a Directories

1. You may wish to remove certain concentrations from your bin datasets to then re-run the hill_analysis routine. You will make a new directory copy of the scripts and prior data to that directory, adding the tag '-reanalysis' to the new working directory. For example, in Cygwin:

```
mkdir 04292014-reanalysis/
cp /cygdrive/e/Eric/04292014/*.* /cygdrive/e/Eric/04292014-reanalysis/.
```

2. You should then edit the the ivbind-merge-script.pl script to include a 'remove' method. First in Cygwin copy the script to a new filename to edit it:

```
cp ivbind-merge-script.pl ivbind-remove-script.pl
```

Then in nano edit the new script:

```
nano ivbind-remove-script.pl
```

Here are the edits to make in nano (yellow highlight):

```
use Ivbind;
my $iv = Ivbind -> new();
$iv -> delimiter(',');
$iv -> open_bin_dir( $ARGV[0] ) -> remove('CONC', [0, 20]) -> save_bins ->
hill_analysis;
```

The modification/insertion is highlighted in yellow. You simply list the concentrations in the square brackets that are to be purged from the bins. Save the file in nano and exit.

This modification allows a directory to be opened and the bin files to be scanned for matches in the 'CONC' column to the values in the square brackets.

3. Execute the perl script on the target directory.

```
perl ivbind-remove-script.pl 04292014-reanalysis/
```

4. Follow steps 5-9 in **Processing a Single Directory** above to load your data into your ORIGIN project.

C. Merging and Removing Concentrations from Bin Datasets at the Same Time

1. Of course the above two procedures may be combined. Make a directory for merging as described above in **(A)**.
2. Copy the scripts to the merge directory and copy and edit the ivbind-merge-script.pl

```
cp ivbind-merge-script.pl ivbind-merge-remove-script.pl
nano ivbind-merge-remove-script.pl
```

3. Here are the recommended edits:

```
use Ivbind;
my $iv = Ivbind -> new();
$iv -> delimiter(',');
$iv -> merge_bin_dirs( [ @ARGV ] ) -> remove('CONC', [0, 20]) -> save_bins ->
hill_analysis;
```

The modification/insertion is highlighted in yellow. Include the list of concentrations in the square brackets.

4. Execute the perl script on the target directories, and they will be merged and purged of particular concentrations of peptide:

```
perl ivbind-merge-remove-script.pl ../04112014/ ../04212014/
```

5. Follow steps 5-9 in **Processing a Single Directory** above to load your data into your `ORIGIN` project.