



## Introduction

This checklist is made to speed up data analysis in an ordered manner.

## Cleaning data

1. Load the result file (\*.blr) of the experiment
2. Remove all the unwanted lines
3. Make a graph of the lines
4. Zero in Y and X
5. Remove unwanted parts of the sensorgrams
6. Remove air spikes
7. Discard the graph
8. Rename the lines when necessary
9. Save the data in a file (\*.ble)

## Subtracting reference line

10. Select lines of one cycle and make a copy
11. Make a graph of the copied lines
12. Subtract the reference line
13. Discard the graph
14. Remove the subtracted line
15. Rename the lines when necessary
16. Save the data in a file (\*.ble)

## Data analysis

17. Select the lines to analyze together and make a copy
18. Make a graph of the lines
19. Select the fitting parts
20. Choose a model
21. Do a fitting
22. Check the results for consistency
23. Give the plot a name
24. Add a legend
25. Save the data in a file (\*.ble)



## SPR analysis checklist (topic explanation)

This checklist is made with the software of Biacore in mind. Other programs such as Scrubber can be used to clean and analyze the data.

### Cleaning data

1. Load the result file (\*.blr) of the experiment  
For the BiaEvaluation software is important to check the box "Use original colors" to give each flow channel its own color. Without the check each line will get a different color.
2. Remove all the unwanted lines  
By removing the lines which do not contribute to the analysis, the dataset will become more organized and the file size smaller.
3. Make a graph of the lines
4. Zero in Y and X  
Select a small timeframe just before the injection of interest and zero in Y. Then place the crosshair at the beginning of the injection and zero in X.
5. Remove unwanted parts of the sensorgrams  
Remove all the parts of the sensorgram at the beginning and end which not contribute to the analysis, like stabilization parts, washing and regeneration.
6. Remove air spikes  
Remove air spikes by making the points invalid. Remove the smallest portion possible.
7. Discard the graph  
This graph is used only for cleaning the data. Removing all the intermediate graphs will reduce the file size.
8. Rename the lines when necessary  
Renaming the lines with descriptive names can speed up analysis and will make the legend more clear.
9. Save the data in a file (\*.ble)  
It is better to give the evaluation file the same name as the result file. The file extension will show the difference.

### Subtracting reference line

10. Select the lines one cycle and make a copy  
A copy of the lines will make it easier to revert to the original lines.
11. Make a graph of the copied lines  
Subtract the reference line
12. Subtract the appropriate reference line and observe the result. It may be necessary to undo the subtraction and realign the lines better to obtain the best reference subtraction.
13. Discard the graph  
This graph is used only for cleaning the data. Removing all the intermediate graphs will reduce the file size.
14. Remove the subtracted line  
The subtracted line is zero and contains no information any more.
15. Rename the lines when necessary  
Renaming the lines with descriptive names can speed up analysis and will make the legend more clear.
16. Save the data in a file (\*.ble)  
Just save in the same file.

### Data analysis

17. Select the lines to analyze together and make a copy  
A copy of the lines will make it easier to revert to the original lines. In addition, grouping corresponding lines will make the analysis more rapid.
18. Make a graph of the lines
19. Select the fitting parts



Select the appropriate curve parts. It is important not to include irregular parts of the curve and setting begin and end of the injection time properly.

20. Choose a model  
Choosing a model should be based on the experiment. So, when a one to one binding is expected, choose this model. Just trying to get the best fit with the different models is not a scientific way of analyzing data. Give the necessary constants and initial values.
21. Do a fitting  
Repeat this with several initial values to obtain the best fit. Different initial values may give different results, due to the algorithms used.
22. Check the results for consistency  
Check whether the values biological relevant and consistent with data obtained in other experiments.
23. Give the plot a name  
The name of the plot should be descriptive about the experiment done.
24. Add a legend  
Adding a legend can be of help when interpreting the graph. A descriptive name to each line is necessary.
25. Save the data in a file (\*.ble)  
Just save in the same file.

## References

1. BIACORE AB; BiaEvaluation 3.0; 1997
2. BIACORE AB; Kinetic and affinity analysis using BIA - Level 1; 1997
3. BIACORE AB; BIACORE Getting Started; 1998
4. BIACORE AB; Kinetic and Affinity analysis using BIA - Level 2; 1998

## Suggested readings

5. O'Shannessy, D. J.; Determination of kinetic rate and equilibrium binding constants for macromolecular interactions: a critique of the surface plasmon resonance literature; *Curr.Opin.Biotechnol.*; (5): 65-71; 1994
6. O'Shannessy, D. J. and Winzor, D. J.; Interpretation of deviations from pseudo-first-order kinetic behavior in the characterization of ligand binding by biosensor technology; *Anal.Biochem.*; (236): 275-283; 1996
7. Ober, R. J. and Ward, E. S.; The Influence of Signal Noise on the Accuracy of Kinetic Constants Measured by Surface Plasmon Resonance Experiments; *Anal.Biochem*; (273): 49-59; 1999
8. Roden, L. D. and Myszka, D. G.; Global analysis of a macromolecular interaction measured on BIAcore; *Biochem.Biophys.Res.Commun.*; (225): 1073-1077; 1996

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