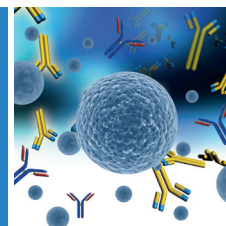


Surface
Plasmon
Resonance
imaging

Quick and easy analysis of hundred bindings based on the interactions clustering, thanks to Cloud Display



Technical Note
Biology
SPRi TN09

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When an experiment is dealing with more than one hundred interactions in a single biochip, the data analysis becomes extremely time consuming. Having a tool to help select the interesting curves from the hundred is very useful. The Cloud Display tool in the EzAnalysis software is the perfect solution, helping to cluster the different interactions according to the type of association / relative dissociation. We will show through two concrete cases, that the Cloud Display is an essential tool to analyze long experiences containing several hundred of interactions and to take full benefit of the multiplex interactions of our system in just one click.

Keywords: High throughput analysis, Affimer/protein interaction, Surface Plasmon Resonance imaging, Molecular interaction clustering

Introduction

EzSuite software is dedicated to analyzing the data generated by the XelPlex system. Its friendly use interface makes it quick to master. The big advantage of using our SPRi systems is the multiplex capability which is the perfect approach to screen many proteins or antibodies, and for clustering many interactions.

When an experiment is dealing with more than one hundred interactions in a single biochip, the data analysis becomes extremely time consuming. Having a tool to help select the interesting curves from the hundred is very useful. The Cloud Display tool in the EzAnalysis software is the perfect solution, helping to cluster the different interactions according to the type of association / relative dissociation. In just one click you can extract a group of binding among hundreds of kinetic curves.

This technical note deals with the great capacity of the Cloud Display to analyze several interactions in one click. This tool will be highlighted with two concrete cases. The EzSuite includes 3 different components (Figure 1):

1. EzView, which is used for monitoring the SPRi experiment;
2. EzAnalysis for data processing;
3. EzFit for the affinity study.

Besides visualization options (injections, kinetic curves, images), the kinetic analysis window gives access to three main tools (Figure 2): Statistic (1), Cloud (2) and Overlay (3). This technical note focused on the cloud tab and shows the power of such option.



Figure 1: EzSuite starting screen



Figure 2: Kinetic analysis screen

The Cloud tab

1. What is it the Cloud ?

It is not a visible mass of condensed water vapor floating in the atmosphere, nor is it a network of remote servers hosted on the Internet. The Cloud is a powerful tool that HORIBA has developed, which allows you to quickly cluster the different kinetic curves in a cloud of points where:

1. Abscissa represents the relative dissociation of binding between your Analyte/Ligand expressed as a percentage;
2. Ordinate is the value at the end of the association expressed in ReS.

Finally, to summarize, each kinetic curve is translated by one point on the graph.

2. How does the cloud work?

It is a breeze to get a transcription of your data in the Cloud graph. When you are in the “kinetic analysis screen”, two values are needed to quickly generate a Cloud graph (Figure 3):

1. The value of “end of association” is indicated by the red cursor. This cursor is fixed automatically and cannot be moved.
2. The “User defined” time indicated by the green cursor is used to calculate the relative dissociation. This cursor is flexible and could be anywhere after the end of association.

$$\text{Relative dissociation} = 1 - (\text{Green cursor value} / \text{Red cursor value}) \times 100$$

Once you have fixed the cursor on the graph, the cloud is drawn instantly and automatically under the kinetic curves graph. On the abscissa axis, the relative dissociation is expressed in %, and on the ordinate axis, the value at the end of the association in ReS unit.

Your graph will be adjusted as soon as you change the user-defined cursor position. The answers obtained and shown in the Cloud graph are subtracted with the negative control already defined. Without negative control definition the cloud graph won't display.

In the cloud graph, each point represents a spot on your biochip, thereby we can see the scattering between the different spots immobilized on the biochips within the same family.

3. What does the Cloud bring me?

Three main pieces of information can be collected from the Cloud graph to help users quickly compare and differentiate their molecules.



Figure 3: Kinetic Cloud analysis

1. On the same graph, we can get the response for each spot and compare them.
2. We can also evaluate the reproducibility (or not) of the responses within the same family. The smaller your cluster is in the Cloud graph, the better your reproducibility is.
3. We can also have an overview of the compound's dissociations during the experiment, thanks to the relative dissociation value calculated and expressed on the Cloud graph on the X axis.

Figure 4 is an example of information that we can obtain thanks to the Cloud tool.

In this case, we can see that the red family has a higher response than the other families, and that its reproducibility is similar to the pink one.

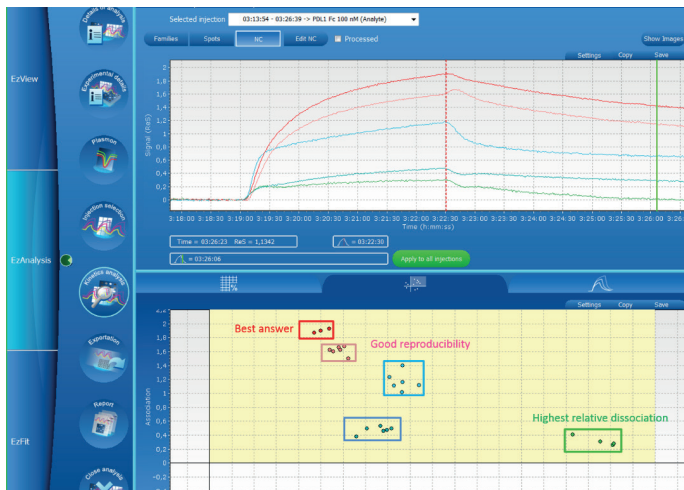


Figure 4: Example of information obtained, thanks to the Cloud tool

4. Cloud options

The Cloud tab offers three option buttons (Figure 5):

- 1.Settings: To display a legend and adjust the setting of each axis;
- 2.Copy: You can paste the graphic directly in another document;
- 3.Save: The Cloud graph can be saved as an “.emf” format.

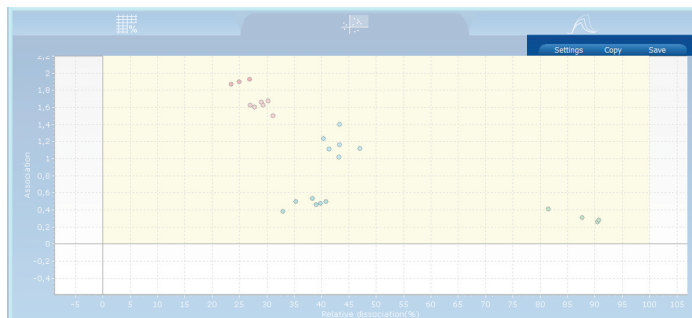


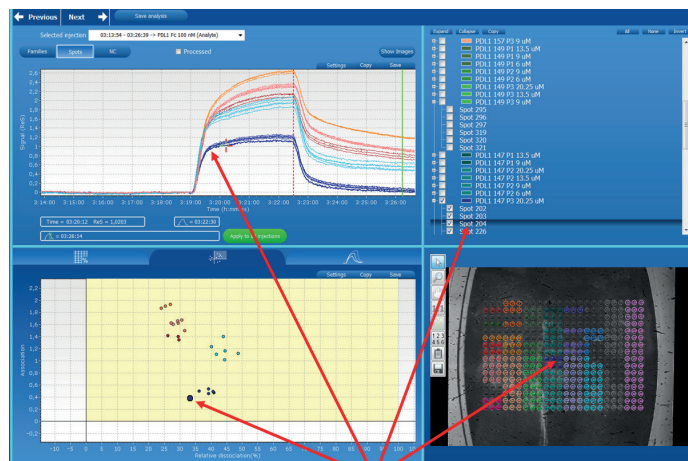
Figure 5: Cloud tab options

By default, all the spots are considered for the measurement's calculation. To get optimum analysis, you can use the “**CV criteria**” parameter. In this case, you can select between 3 degrees of coefficients of variation: **Weak**, **Medium** or **High** in the drop-down menu in the “statistic tab”. This allows you to exclude one or more points that are too far from the mean. The removed spots are labelled by red squares as shown in Figure 6. Weak, Medium and High correspond to the exclusion level applied within the same family.



Figure 6: CV criteria application

Moreover, in the EzAnalysis software, there is a permanent link between the different parts of the kinetic analysis window. Thus, when you select a point on the cloud graph, its corresponding spot on the biochip and its corresponding kinetic curves are highlighted (Figure 7).



Highlighting the spot 204, shows its curve, its position on the biochip and on the cloud graph.

Figure 7: Highlighting a spot

Case studies

We will highlight the processing power of the cloud through two screening application examples: Affimer/protein and antibody / protein interactions. The understanding of the data and the candidate selection depends on the application and the experiment conditions performed.

1. Affimer/protein interaction

Affimers are small single domain proteins (98aa, 10kDa, without inserts) designed to mimic the molecular recognition capabilities characteristic of monoclonal antibodies in different applications. They are based on the Cystatin family protein. Interactions are generated through the insertion of random sequences in loops 2 & 4 (see Figure 8).

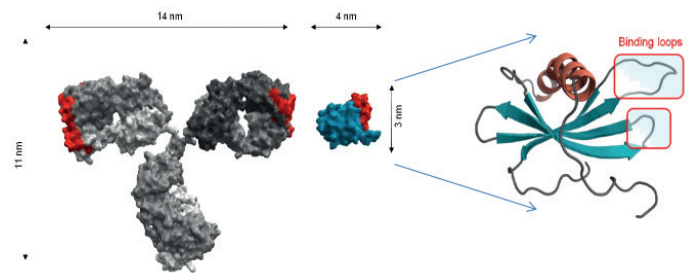


Figure 8: Affimer alternative to antibodies as affinity reagents

Moreover, the affimers represent many advantages such as:

- Rapid development: Save time, get custom reagents in weeks instead of months;
- Engineered specificity: Design your screening process to select desired binding properties;
- Flexible functionalization: Easily format affimer sequences to maximise performance in multiple applications and assays;

- Ease of manufacturing: Consistent and cost-effective productions, using simple bioprocesses;
- Not limited by immune system: Enable difficult targets that would be challenging for antibody technologies.

1.1. Aim of experiment

The aim of this first application is to define the best spotting condition and the best candidate between six affimers. To do so, affimers were immobilized at three different pH conditions (4, 5 and 7.4) and at four concentrations (6, 9, 13.5 and 20.25 μ M), leading to 58 different spotting conditions and 384 spots (Figure 9).



Figure 9: Image of the spotted biochip

1.2. Results

A target protein was injected on the biochip and 384 sensorgrams were generated (see Figure 10). We have applied the Cloud tab tool on those sensorgrams (Figure 11), causing some immobilization conditions to stand out from the rest.

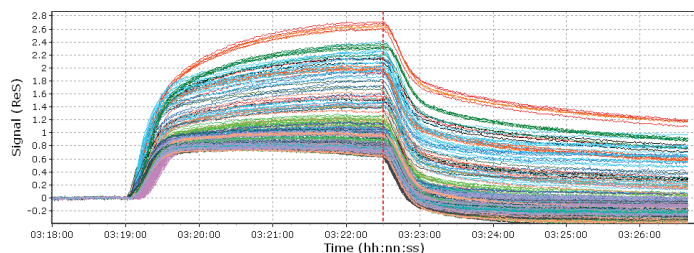


Figure 10: Sensorgrams generated after injection of the target protein

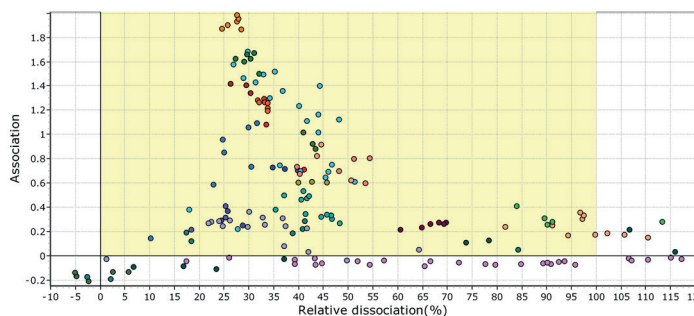


Figure 11: Sensorgrams converted, thanks to the Cloud graph

To increase the readability of the data, we will process the analysis in two steps: First, we will analyze each Affimer separately to identify the best condition of immobilization. Then we will determine the best aptamer based on the optimal condition for each one.

Figure 12 shows the results observed for the affimers PDL1. By using the Cloud, we can determine that the best condition to immobilize the affimer PDL1 141 is 13.5 μ M at pH5. This condition corresponds to the highest association signal and the lowest relative dissociation, compared to the other immobilization conditions.

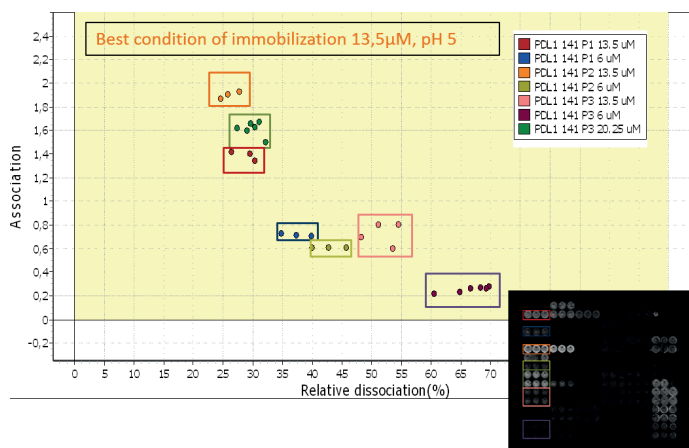


Figure 12: Responses comparison obtained for different immobilization conditions for affimer PDL1 141

Once the best immobilization condition for each affimer is determined, we proceed to the identification of the best candidate between the different affimers.

We have added in the same Cloud graph (see Figure 13) all the best immobilization conditions for each of them.

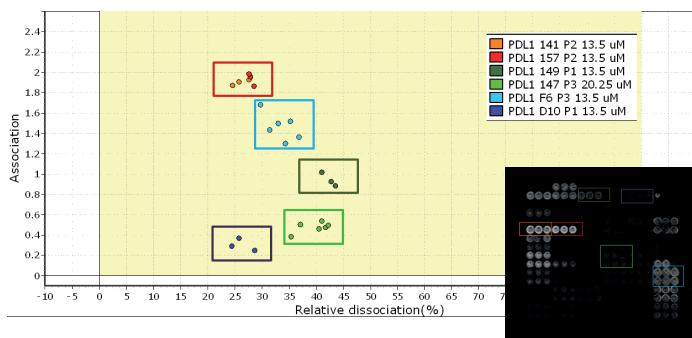


Figure 13: Responses obtained for six different affimers in their best immobilization conditions

We can see that all affimers have approximately the same relative dissociation (Between 25 and 40%) but the best candidates are the affimers 141 and 157, since they show the highest response (~2 ReS).

2. Antibodies/protein interaction study

2.1. Purpose of the experiment

The purpose of this experiment using surface plasmon imaging technology is to study the interaction between virus envelope protein and some antibodies. Using this model, we will illustrate the use of the Cloud tab from an experiment where we will be able to rank ten different antibodies.

The ten antibodies were prepared in 10mM PBS pH 7.4 buffer and immobilized in quadruplicate at a concentration of 7 μ M.

2.2. Results

Kinetic data obtained after injection of virus envelope proteins at 50nM are illustrated below (Figure 14).

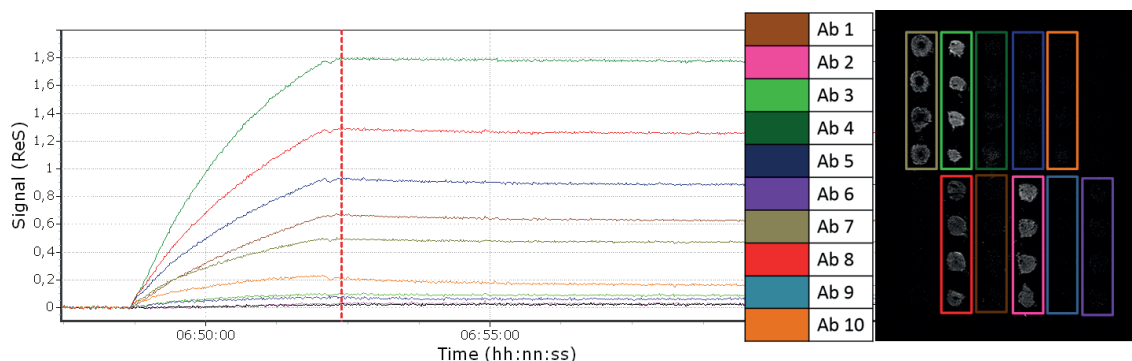


Figure 14: Graphic obtained after injection of the protein

We can make a clear distinction between antibodies according to their ability to bind to the protein.

As shown in Figure 15, we can classify them into 3 different groups: low, medium and high binders.

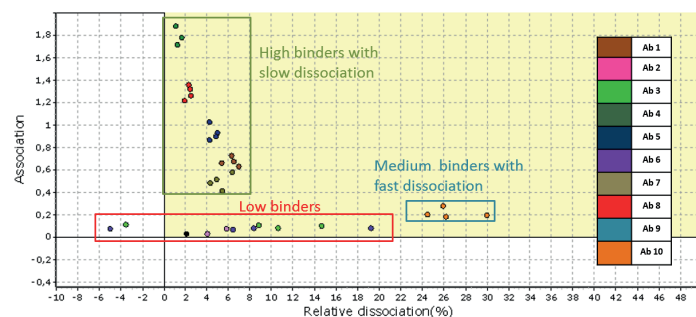


Figure 15: Three different clusters can be highlighted, thanks to the data Cloud display

This rapid differentiation between families allows us to remain focused on the high binders that are the most interesting clusters for the rest of our application.

Conclusion

We have shown in this technical note through two applicative examples, how the Cloud option can be very useful to:

- Evaluate in one click, molecules binding with different kinetic parameters (based on the association and the relative dissociation) among hundreds of kinetic curves;
- Cluster antibodies according to their affinity and their relative dissociation;
- Observe the responses' reproducibility between all spots within each family;
- Get a quick analysis of raw data and optimize your research according to the molecule profiles observed.

All these advantages will help you to make a quick analysis of your generated data.