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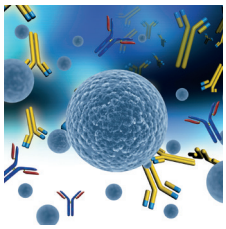
Surface Plasmon Resonance imaging

Comparison of kinetic curves for multiple analytes in one click highlighted by the Overlay Tool of EzAnalysis software for XelPleX system Examples of crude samples analysis



Technical Note
Biology
SPRi TN07

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The fully automated XelPleX system is dedicated to high-throughput analyses of biomolecular interactions generating a considerable amount of data. This type of high performance system needs specific processing tools to quickly extract the valuable information. The overlay display is one powerful tool of the EzAnalysis software provided with the XelPleX system. It displays an instant comparison of kinetic curves, either of multiple analytes, or of one analyte injected in different conditions, for each immobilized ligand. This feature is illustrated by different application examples analyzing crude samples.

Keywords: Multiplex Comparison, High-throughput Analysis, Crude samples, Surface Plasmon Resonance imaging (SPRi).

Introduction

A crude sample is a sample that exists in a natural state and is unaltered by any process or any dilution. Characterizing molecular affinities in the native medium without purification or modification, makes it possible to fully understand the biological mechanisms at the molecular scale.

Our XelPleX system allows the analysis of the molecular binding in a complex media and generates valuable data, such as the determination of real-time physico-chemical interactions and their kinetics.

This technical note highlights the advantage of the overlay display, which allows fast and simple comparison of multiple kinetic curves registered at different times. It gives a quick overview of all the selected kinetic curves in just one click. Thus, the users performing high throughput applications will get the most advantage of the overlay option.

General description of the EzSuite software

The XelPleX system is powered by the EzSuite software. The EzSuite software offers fast and easy kinetic interaction analysis, from data monitoring to the generation of a final report. The EzSuite includes 3 different components (Figure 1):

1) EzView to monitor your SPRi experiment;
2) EzAnalysis for quick processing of the data set registered during your SPRi experiment; and
3) EzFit for the advanced analysis of the affinity constants.

Figure 1: EzSuite starting screen

Detailed description of the overlay tool

In this technical note, the overlay tool of EzAnalysis will be highlighted. The overlay is a display option of the kinetic curves for one specific ligand family. The overlay display corresponds to Tab 3 of the kinetics analysis window (Figure 2). The first tab gives access to statistic tables. The statistics tab is a quantitative analysis tool; its use is described in our technical note, TN n°6 "EzAnalysis to accelerate proteins-based applications". Tab 2 is the cloud display option to cluster the ligands according to their binding responses to an analyte, and the relative dissociation of the complex. The cloud display helps perform the ranking of numerous ligands.

Figure 2: Kinetic analysis window

The aim of the overlay tab is to superimpose the kinetic curves obtained during different injections (at different times) for one ligand family. The overlay can be applied to non-processed curves only. The tick box “A” of Figure 3 must be unchecked. Processed curves mean that some spots are excluded manually or automatically (through the use of CV criteria in the statistics tab).

The visualization for one ligand family can be:
1) Per family (averaged curves);
2) Per spot; and
3) Per negative control referenced family (averaged and reference-subtracted curves). See Figure 3.

Figure 3: General overview of the overlay tab

The ligand of interest is defined in the dropdown list, “Family”, as shown in step B in Figure 3.

When the “spots display” function is used for the overlay, a second dropdown list is available. This list corresponds to the spots belonging to the selected family. Spot(s) can be easily removed from the overlay graph by unselecting the corresponding tick box(es), as illustrated by step C of Figure 4.

Figure 4: Detailed overlay tab

The displayed injections are all the injections selected in the previous step, “injection selection”, of EzAnalysis. Thus, the choice of the injections that the user wants to superimpose using the overlay tool must be defined in the step, “Injection selection”, (Figure 5, step 1). Also, the colour of the curves is set for each selected injection in the list of injections in this step. (Figure 5, step 2).

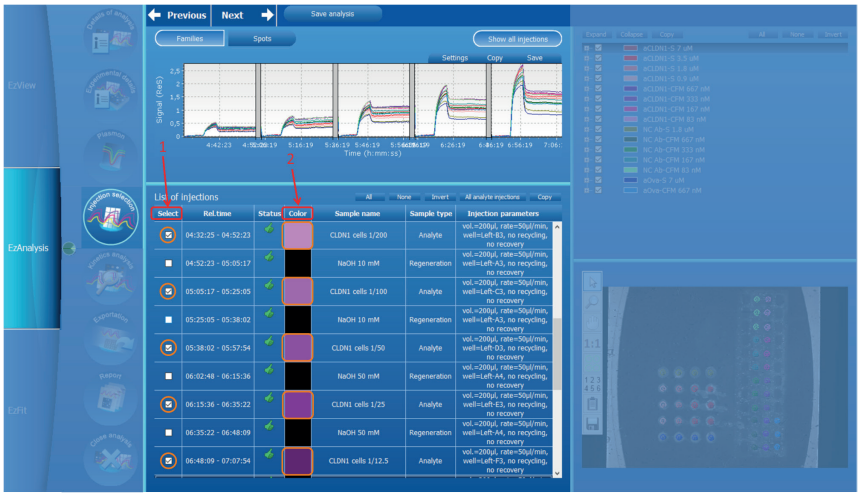

Figure 5: Injection selection screen

The overlay graph can be exported under different formats (Figure 6):

1) Legend and scale settings: A legend can be added. Different positions around the graph are suggested.
2) A copy/paste of the graph can be easily done.
3) The graph data can be exported in a text format.
4) The graph can be saved as an image format.

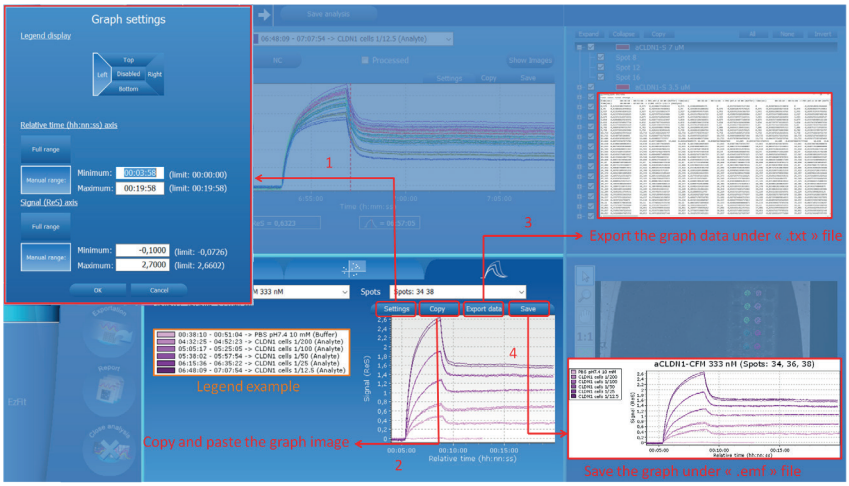

Figure 6: How to export the graph.

Application examples

1. Injections of cell culture at increasing concentrations

In this first application example, an evaluation of the affinity was to be considered. The studied interaction concerns an antibody and a transmembrane protein expressed at the cell surface. The specific antibody was immobilized on a SPRi-Biochip™ in triplicate. The transfected cells were injected at 5 increasing concentrations following a two-fold dilution series (the initial solution was diluted at 1/200, 1/100, 1/50, 1/25 and 1/12.5).

Figure 7 shows the kinetic curves obtained for the 3 spots of the specific antibody after the injections of the transfected cells at different dilutions. The curves corresponding to the different cells' dilutions injected at different times are superimposed, thanks to the EzAnalysis overlay tool.

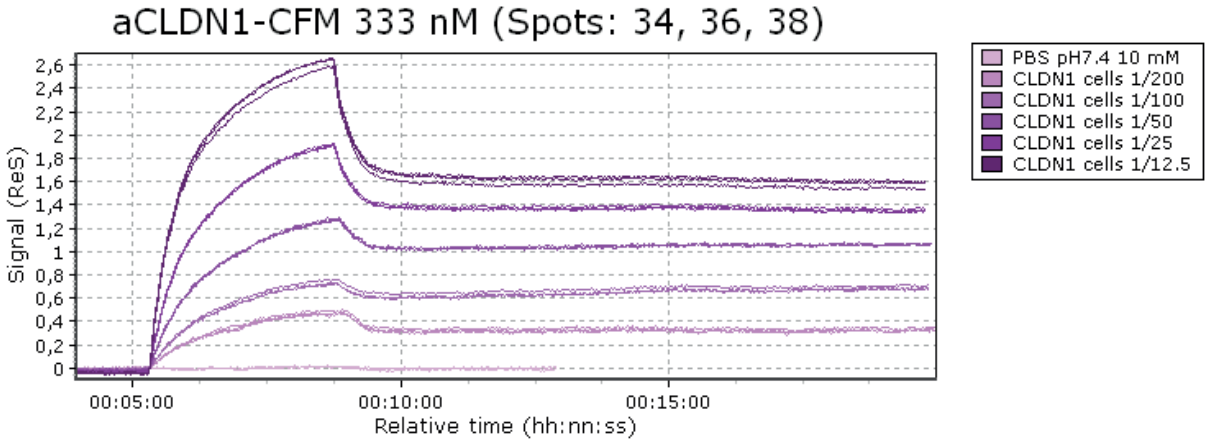

Figure 7: Kinetic curves obtained for the specific antibody spots immobilized under specified conditions after the injections of cells at different dilutions. The curves corresponding to the different cells dilutions injected are superimposed.

This visualization mode allows easy and fast observation of the homogeneity among the different spots replicates. It also gives the opportunity to visualize all the kinetics of interest in few seconds, in one click.

2. Comparison of different sera samples

The context of this second application example is the characterization of the antibody response of milk allergic patients (work performed in collaboration with Dr. Hélène Chardin, CNRS-ESPCI, Paris, France). 3 major milk allergens (α-lactalbumin, β-lactoglobulin and casein) were immobilized on an activated SPRi-Biochip™ CH-HD using the SPRi-Continuous Flow Microspotter (SPRi-CFM). The printed SPRi-Biochip™ was then inserted into the XelPlex™ system. 7 sera of milk allergic patients were injected at different dilutions.

Figure 8 compares the kinetics curves obtained on the casein spots after the injection of the 7 sera diluted at 1/125. These kinetics curves are averaged and reference-subtracted curves. Figure 8 is extracted from the overlay of EzAnalysis.

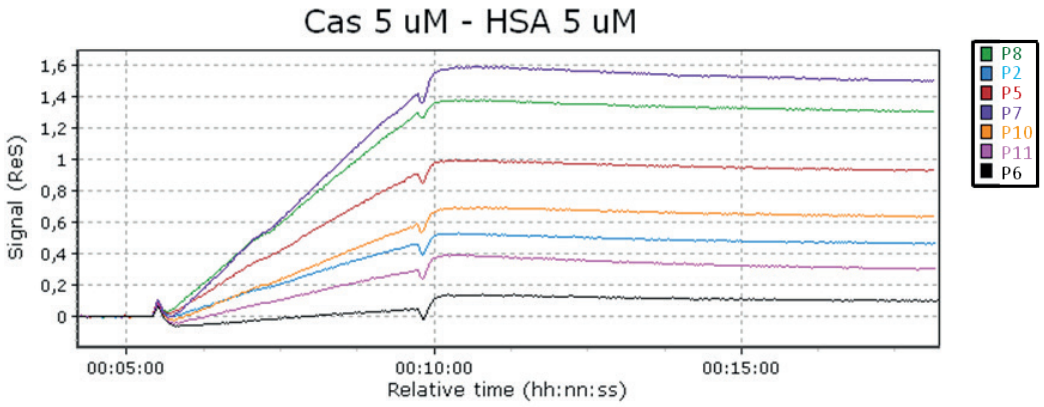

Figure 8: Comparative analysis of the antibody response of several patients to the casein allergen

This kind of overlay figure is very quickly generated with the EzAnalysis software, and allows efficient comparison of the immunoglobulin contents of various sera for one allergen. In this example, the comparison shows that the quantity of antibodies produced against the casein allergen may vary from 1 to 15 times from one patient to another. As an example we noticed that casein is more immunogenic for patient P7 than for patient P6.

Conclusion

In this technical note, the powerful data processing feature of EzAnalysis was demonstrated through the overlay option. This technical note is in addition to 2 other technical notes describing the statistics functions and the cloud display of EzAnalysis, respectively. The overlay display enables an instant comparison of kinetic curves, either of multiple analytes, or of one analyte injected in different conditions, per each immobilized ligand.

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