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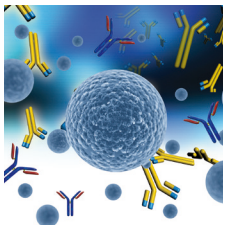
Surface Plasmon Resonance imaging

Two case studies using EzAnalysis to accelerate proteins-based applications



Technical Note
Biology
SPRi TN06

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Introduction

EzSuite software is dedicated to the analysis of data generated on the XelPleX system, which accelerates proteins-based applications. Its friendly user interface makes it quick and easy to master.

EzSuite includes 3 different components (**Figure 1**):
1) EzView, which is used to monitor the SPRi experiment,
2) EzAnalysis for data processing,
3) EzFit for the affinity study.

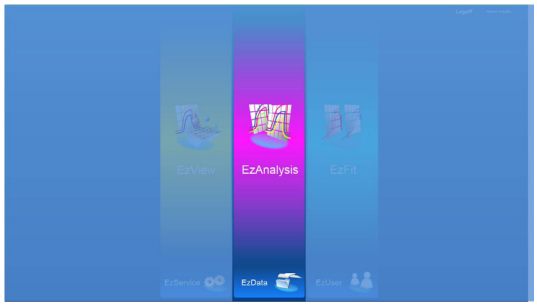


Figure 1: EzSuite starting screen

This technical note addresses the use of the kinetic analysis tools, and is focused on the EzAnalysis part of the EzSuite. Besides visualization options (injections, kinetic curves, images), the Kinetics analysis window gives access to three main tools (**Figure 2**): Statistics (1), Cloud display (2) and Overlay (3). This technical note will focus on the Statistics tab.

Figure 2: Kinetics analysis screen

The Statistics tab displays the kinetic measurements performed during the SPRi experiment at two different times during the injection (**Figure 3**):

1- The "**end of association**" is indicated by the red cursor
2- The "**User defined**" time is indicated by the green cursor.

Figure 3: Kinetic measurement times

For each time, 2 values are shown in the statistics table: ReS (Resonance Shift) and pg/mm2. These values are automatically calculated for each spot, for each family (average of several spots) and for each negative control subtracted family (**Figure 4**).

Figure 4: Statistic's table values

The statistics table can be saved as a ".txt" format using the "save button", or pasted in an Excel file using the "Copy" button **(Figure 4)**. By default, all the spots are taken into account for the measurements calculation. "**CV criteria**" can be applied by selecting **Weak**, **Medium** or **High** in the drop-down menu. The software will exclude one or more curves that are too far from the mean. If a curve is removed, an exclamation mark appears in front of the family name. The color depends on the total number of spots and on the proportion of the suppressed spots **(Table 1)**.

		Number of family spots		Proportion of spots removed
No indicator		>2		0%
Green		>2	and	≤25%
Orange	or	<2		≤25%
		>2		> 25%
Red		∀		100%

Table 1: Statistic's table indicators

Figure 5 shows an overview of the impact of the CV criteria choice on the statistics table.

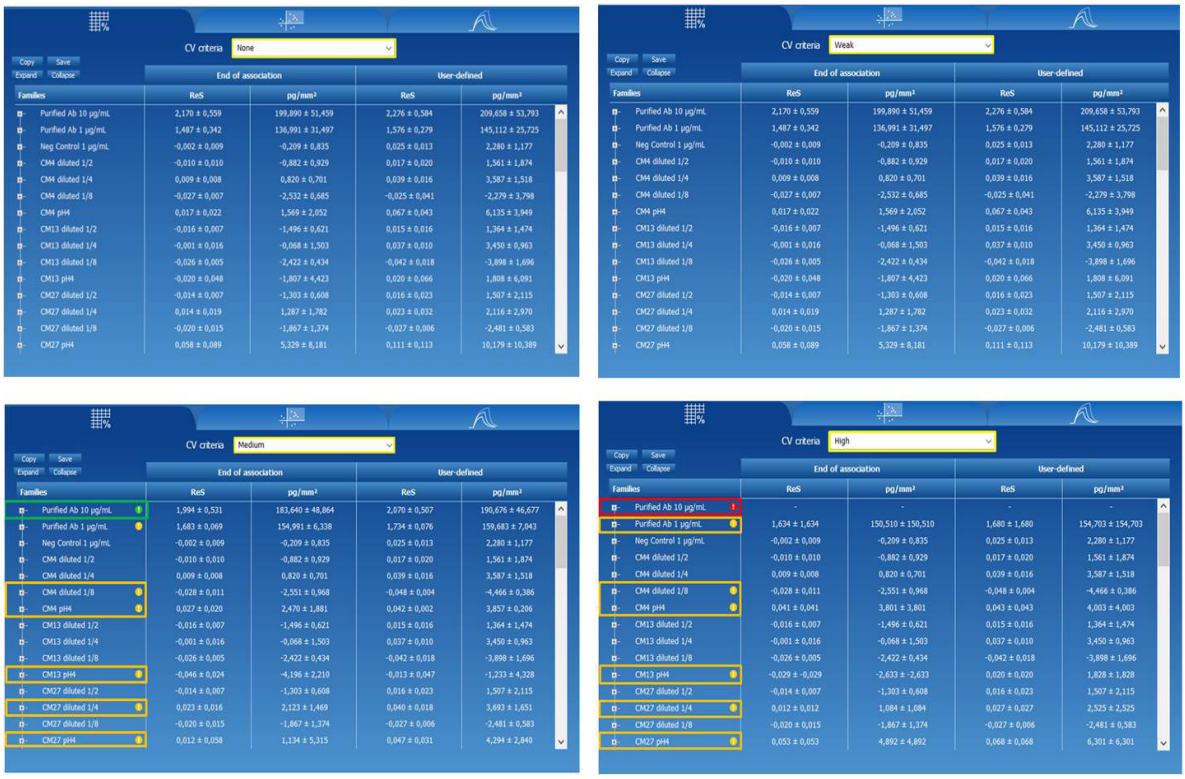


Figure 5: CV criteria indicators

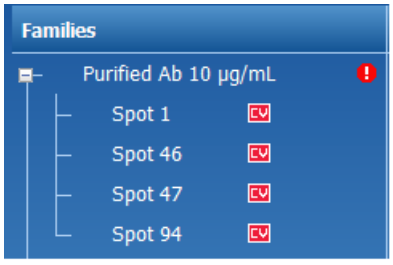


Figure 6: Removed spots

The removed spots are labelled by red squares as shown in the **Figure 6**.

Applicative examples

1. Experimental conditions optimization

We will illustrate the use of the statistics option with an application example. For this demonstration, we performed a protein/antibody interaction study in order to define the best candidate and the best spotting condition. To do so, 3 different antibodies were immobilized at 4 different concentrations: 0.25 nM, 0.5 nM, 0.75 nM and 1 nM. Then the protein was injected. The kinetic data exported from EzAnalysis are shown in **Table 2**.

		End of association				User-defined			
		ReS	Std. deviation	pg/mm²	Std. deviation	ReS	Std. deviation	pg/mm²	Std. deviation
mAb1	1 nM	-0,034	0,011	-3,157	1,02	-0,03	0,013	-2,793	1,161
	0,75 nM	0,012	0,005	1,064	0,431	0,019	0,007	1,778	0,602
	0,5 nM	-0,02	0,017	-1,8	1,56	-0,003	0,015	-0,271	1,369
	0,25 nM	0,009	0,02	0,87	1,848	0,025	0,019	2,319	1,778
mAb2	1 nM	0,86	0,107	79,177	9,84	0,853	0,088	78,54	8,06
	0,75 nM	0,947	0,082	87,252	7,515	0,949	0,087	87,362	7,975
	0,5 nM	1,035	0,056	95,278	5,176	1,037	0,057	95,529	5,242
	0,25 nM	1,392	0,242	128,207	22,273	1,395	0,238	128,512	21,945
mAb3	1 nM	-0,053	0,022	-4,927	2,02	-0,05	0,022	-4,602	2,015
	0,75 nM	0,024	0,01	2,221	0,927	0,023	0,014	2,159	1,301
	0,5 nM	-0,026	0,002	-2,4	0,225	-0,017	0,003	-1,603	0,277
	0,25 nM	-0,017	0,016	-1,563	1,478	-0,008	0,012	-0,77	1,123

Table 2: Exported statistics data

The following histogram (**Figure 7**) shows the responses for each condition, as well as the standard deviation (SD) for each interaction. This SD is calculated based on the average of the different spots for the same species. We can easily and quickly see that the best candidate is mAb2 spotted at 0.25nM.

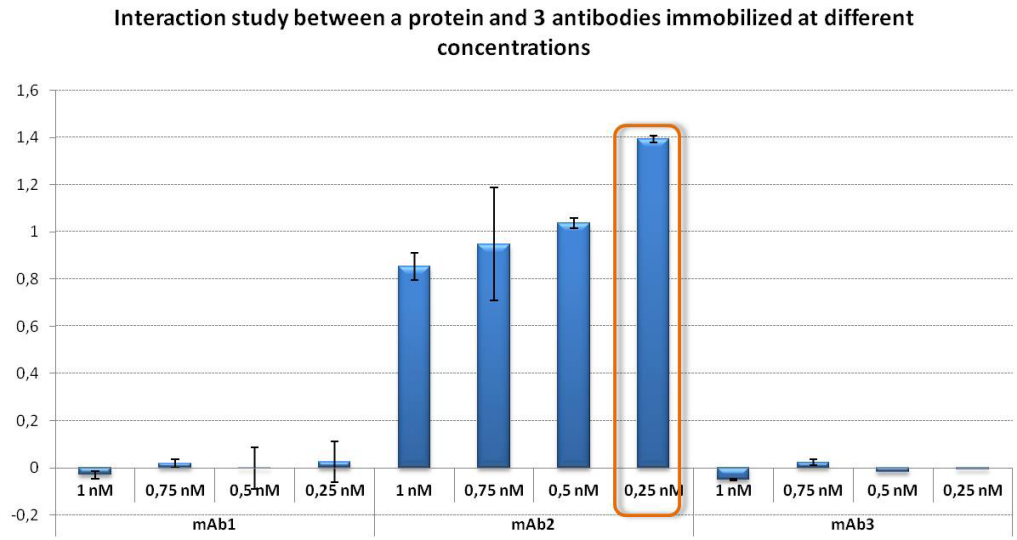
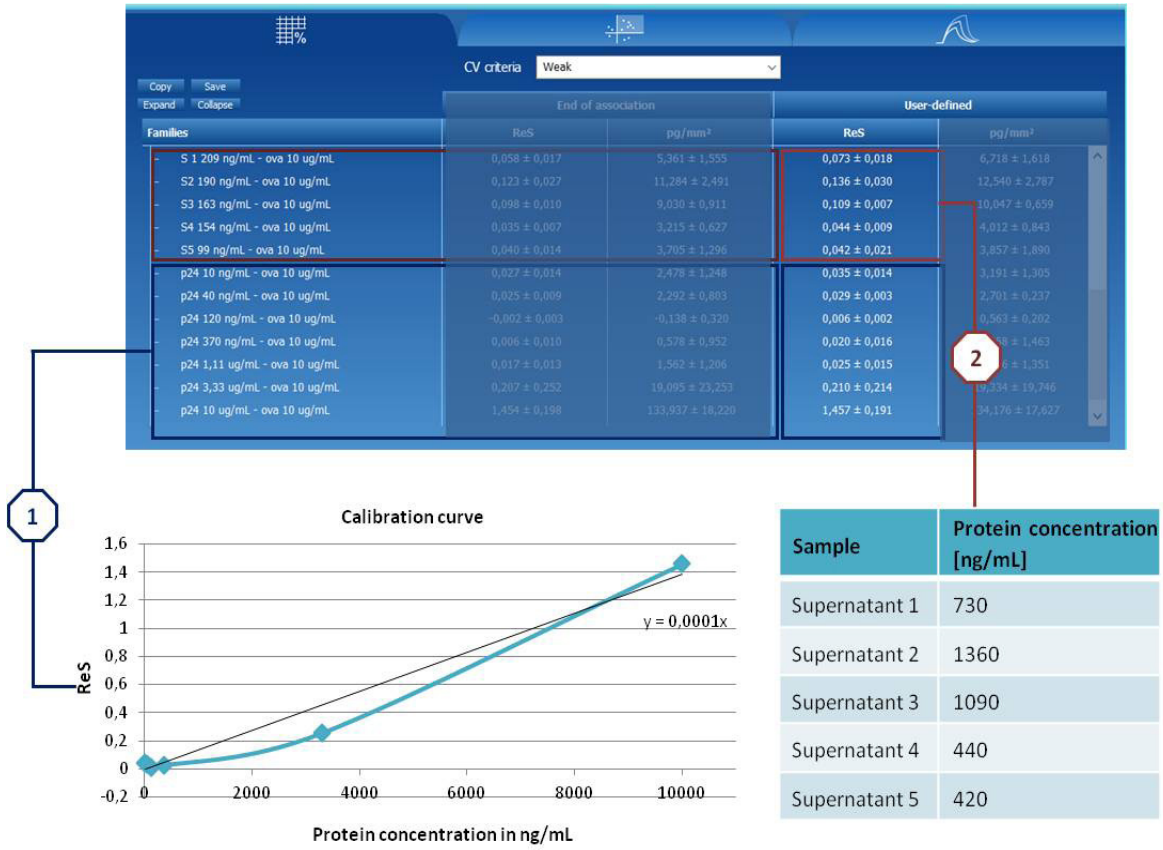


Figure 7: Responses measured between three antibodies (mAb1, mAb2 and mAb3) immobilized at 4 different concentrations: 0.25 nM, 0.5 nM, 0.75 nM and 1 nM and the injected protein.

2. Protein quantification in crude samples

The aim of this application is to determine the protein concentration in different crude samples. First, the protein was captured at increasing concentrations using a primary antibody in order to establish a calibration curve. In parallel, the protein was captured from 5 different crude samples. Finally, one injection of the secondary antibody was performed. The generated data were processed using EzAnalysis, and the protein was quantified, as explained below, and seen in **Figure 8**.



Sample	Protein concentration [ng/mL]
Supernatant 1	730
Supernatant 2	1360
Supernatant 3	1090
Supernatant 4	440
Supernatant 5	420

Figure 8: Protein quantification using a calibration curve

The statistics table shows the responses after injection of the protein at increasing concentrations and the different supernatants: S1, S2, S3, S4 and S5.

The first time, a calibration curve was traced based on the generated ReS values **1**. The second time the calibration curve was used to convert the responses in ReS measured after injection of the supernatants to a protein concentration in ng/mL **2**.

Conclusion

EzAnalysis is a powerful tool that enables a streamlined workflow for data processing. The user friendly and intuitive software interface allows for immediate ease of use. As demonstrated in this technical note, through two different applications, quantitative binding studies are made easy thanks to the EzAnalysis Statistic function by pushing just one single button.