



Surface Plasmon Resonance imaging Two case studies using EzAnalysis to accelerate proteins-based applications



Technical Note Biology SPRi TN06

Fatima-Ezzahra Hibti, Chiraz Frydman, HORIBA Scientific, Palaiseau, France

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

HORIBA

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

HORIBA

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	N 1.9	>2		0%
Green		>2	and	≤25%
0		<2		≤25%
Orange	or	>2		> 25%
Red		V		100%

Table 1: Statistic's table indicators

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

# %				A	# %		<u></u>	T	A
	CV orteria 🛛 No	ne	Ŷ		Treasured In Second	CV orteria Weak 🗸			
Expand Collapse	End of association		User-defined		Expand Collapse	End of association		User-defined	
Families	ReS	pg/mm²	ReS	pg/mm ²	Families	ReS	pg/mm ²	ReS	pg/mm ²
- Purified Ab 10 µg/mL	2,170 ± 0,559	199,890 ± 51,459	2,276 ± 0,584	209,658 ± 53,793 ^	- Purified Ab 10 µg/mL	2,170 ± 0,559	199,890 ± 51,459	2,276 ± 0,584	209,658 ± 53,793
 Purified Ab 1 µg/mL 				145,112 ± 25,725	p- Purified Ab 1 µg/mL		136,991 ± 31,497		145,112 ± 25,725
- Neg Control 1 µg/mL				2,280 ± 1,177	p- Neg Control 1 µg/mL				2,280 ± 1,177
- CMH diluted 1/2		-0,882 ± 0,929		1,561 ± 1,874	p- CM4 diluted 1/2				1,561 ± 1,874
p- CM4 diluted 1/4				3,587 ± 1,518	p- CM4 diluted 1/4				3,587 ± 1,518
				-2,279 ± 3,798	p- CM4 diluted 1/8				-2,279 ± 3,798
				6,135 ± 3,949	p- CM4 pH4				6,135 ± 3,949
				1,364 ± 1,474	CM13 diluted 1/2				1,364 ± 1,474
				3,450 ± 0,963	p- CM13 diluted 1/4				3,450 ± 0,963
				-3,898 ± 1,696	p- CM13 diluted 1/8				-3,898 ± 1,696
				1,808 ± 6,091	0- CM13 pH4				1,808 ± 6,091
				1,507 ± 2,115	p- CM27 diluted 1/2				1,507 ± 2,115
				2,116 ± 2,970	n- CM27 diluted 1/4				2,116 ± 2,970
				-2,481 ± 0,583	p- CM27 diluted 1/8				-2,481 ± 0,583
				10,179 ± 10,389	E- CM27 pH4				10,179 ± 10,389

			Y	A	# %				A	
CV arterio Medum 🗸			<u> </u>		Copy Save	CV criteria Hig	CV orteria High 🗸			
Expand Collapse	End of association		User-defined		"Expand" "Collapse"	End of association		User-defined		
Families	ReS	pg/mm ²	ReS	pg/mm²	Families	ReS	pg/mm²	ReS	pg/mm ²	
p- Punified Ab 10 µg/mL 🕕	1,994 ± 0,531	183,640 ± 48,864	2,070 ± 0,507	190,676 ± 46,677 🔥	🖬 - Punified Ab 10 µg/mL 🚯				· · ·	
🕫 - Purified Ab 1 µg/mL 🛛 🥥		154,991 ± 6,338		159,683 ± 7,043	🛱 - Purified Ab 1 µg/mL 📀				154,703 ± 154,703	
B- Neg Control 1 µg/mL				2,280 ± 1,177	 Neg Control 1 µg/mL 				2,280 ± 1,177	
p- CM4 diluted 1/2				1,561 ± 1,874	p- CM4 diluted 1/2				1,561 ± 1,874	
p- CM4 diuted 1/4				3,587 ± 1,518	 CM4 diluted 1/4 				3,587 ± 1,518	
p- CM4 diluted 1/8				-4,466 ± 0,386	n- CM4 diluted 1/8 🔱				-4,466 ± 0,386	
р- СМ4 рН4 🔍				3,857 ± 0,206	🖬 - CM4 pH4 🚺				4,003 ± 4,003	
D- CM13 diluted 1/2				1,364 ± 1,474	n- CM13 diluted 1/2				1,364 ± 1,474	
				3,450 ± 0,963	p- CM13 diluted 1/4				3,450 ± 0,963	
				-3,898 ± 1,695	p- CM13 diluted 1/8				-3,898 ± 1,696	
🕫- CM13 pH4 🚺				-1,233 ± 4,328	ф- СМ13 рН4 🚺				1,828 ± 1,828	
p CM27 diluted 1/2				1,507 ± 2,115	p - CM27 diluted 1/2				1,507 ± 2,115	
ti - CM27 diluted 1/4				3,693 ± 1,651	n- CM27 diluted 1/4				2,525 ± 2,525	
B- CM27 diluted 1/8				-2,481 ± 0,583	- p- CM27 diluted 1/8				-2,481 ± 0,583	
n- CM27 pH4 🕚				4,294 ± 2,840 🗸	ф СМ27 рН4 🚺				6,301 ± 6,301 🗸	

Figure 5: CV criteria indicators

Families							
-	– Purified Ab 10 μο	g/mL 😶					
	– Spot 1	EV					
	– Spot 46	EV					
	– Spot 47	EV					
	L Spot 94	EV.					

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

HORIBA

Figure 6: Removed spots

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
	1 nM	-0,053	0,022	-4,927	2,02	-0,05	0,022	-4,602	2,015
mAb3	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

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.



Interaction study between a protein and 3 antibodies immobilized at different concentrations

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.

HORIBA

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**.



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.

info.sci@horiba.com

USA: +1 732 494 8660 UK: +44 (0)20 8204 8142 China: +86 (0)21 6289 6060 France:+33 (0)1 69 74 72 00Italy:+39 2 5760 3050Brazil:+55 (0)11 5545 1500

Germany: +49 (0) 6251 8475 0 Japan: +81 (0)3 6206 4721 Other: +33 (0)1 69 74 72 00

www.horiba.com/scientific

HORIBA