

PMI Dose Response Fold Change Report

When you open the oxidative footprinting master workflow, you will see two columns in the Samples table of the Samples tab: "**Sample**" and "**Exposure Time**". Those columns are unique to XFMS and have been incorporated into the workflow for the purpose of supporting the "Dose Response Fold Change" report which is now the fourth tab of the XFMS report template. **Please note that these two columns only apply to a standard, two-state footprinting experiment. They should be left blank for all other projects, including single-peptide work and FLASH experiments.**

Sample name	Sample type	MS file	MS/MS search	Digestion	Sample	Exposure Time
4-800	Reference	D:/P450/051024...			Free	800
4-1000	Reference	D:/P450/051024...			Free	1000
4-0_20ul	Reference	D:/P450/051024...			Free	0
4-200	Reference	D:/P450/051024...			Free	200
4-400	Reference	D:/P450/051024...			Free	400
4-600	Reference	D:/P450/051024...			Free	600
6-400	Reference	D:/P450/051024...			Complex	400
6-600	Reference	D:/P450/051024...			Complex	600
6-800	Reference	D:/P450/051024...			Complex	800
6-1000	Reference	D:/P450/051024...			Complex	1000
6-0	Reference	D:/P450/051024...			Complex	0
6-200	Reference	D:/P450/051024...			Complex	200



Please take these steps in the **Samples table** of the workflow:

- In the "**Sample**" column, designate each sample as either **Free** or **Complex** (see screenshot above). You must use these designations exactly as written (case-sensitive). Other designations will not work. No spaces should be present since a space will be registered as a character in the string.
- Input the numeric exposure time in the "**Exposure Time**" column. Take care to input the correct exposure times for each sample as these values are used in the regression analysis. No spaces should be present since a space will be registered as a character in the string.

After project creation and after having filled in any MS2 gaps using "Add Missing Via Existing Peptides", you can assess the overall quality of the dose response prior to validation by taking a look at the % modification trend across samples in the second report tab, "%Mod by ModName across Samples" which groups data similar to the Dose Response Fold Change report.

Report layout

The Dose Response Fold Change report is based on a linear regression analysis of $\ln(\text{fraction unmodified})$ as a function of exposure time. Fraction unmodified is calculated

based on the simple calculation of XIC Ratio% which is the %Mod figure shown in the Peptides table and in the %Mod by ModName across Samples report. Please note that the Dose Response Fold Change report uses the full precision XIC Ratio% in calculations whereas the %Mod by ModName across Samples report will export %Mod with the number of significant figures shown in the pivot table.

The Dose Response Fold Change report produces the following measures and summary statistics:

Sample ↓	Intercept ↑	Slope	Slope Std. Error	r2 ↑	Fold Change ↑
Free	-0.000253211	-0.000037229462	0.0000023894974	0.933408	0.9359903675494087
Complex	-0.000157669	-0.000039775476	0.000001027738	0.98957	0.9359903675494087

Sample: the **Free** or **Complex** sample series used for analysis based on the designation assigned to each sample in the workflow.

Intercept: the linear regression uses a forced intercept equal to the value of $\ln(\text{fraction unmodified})$ at $x=0$ to account for any background oxidation.

Slope: this is the hydroxyl radical reactivity rate constant. Report the absolute value of this figure and multiply by $1e6$ if the exposures were in μs .

Slope Std. Error: the standard error of the slope ($n - 1$ degrees of freedom since we are not estimating the intercept).

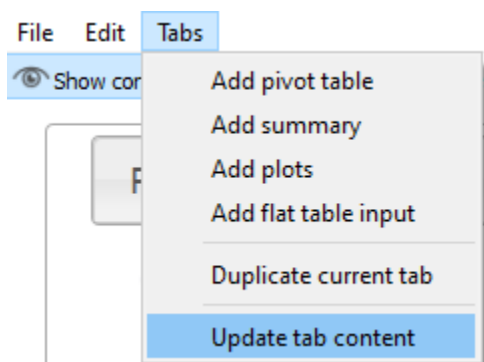
r2: R-squared value

Fold Change: the **Free** hydroxyl radical reactivity rate constant (Slope) divided by the **Complex** hydroxyl radical reactivity rate constant (Slope). The Fold Change figure is shown in both rows due to how the pivot table is structured.

The data values shown for each **Exposure Time** correspond to $\ln(\text{fraction unmodified})$:

Exposure Time	0	200	400	600	800	1000
	-0.0149	-0.0238	-0.0451	-0.0551	-0.0796	-0.0786
	-0.00305	-0.0146	-0.0439	-0.0528	-0.0726	-0.0689

When you have validated your peptides and set the peptides to be included in the final analysis to “True-positive” in the Validate column of the Peptides table, then filter the Peptides table for “True-positive” peptides only. In the Dose Response Fold Change report, please click “Update tab content” to make sure all changes made in the Peptides table are reflected in the report.

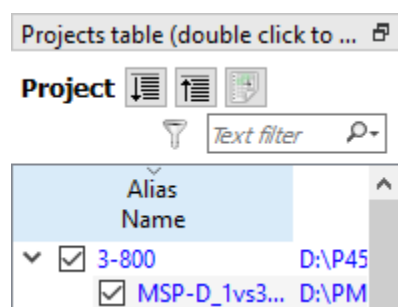


New in-silico peptides created from original peptide

! If you have created a new in-silico peptide based on an original peptide in the Peptides table, please note that the Dose Response Fold Change report will sum the %Mod data for the original peptide and any corresponding new in-silico peptides, regardless of any labels applied to the peptides. This is different than how the same peptides are processed in the %Mod by ModName across Samples report where peptides are only summed if the Labels field is either blank or contains the same label. If you wish to view or report the dose response fold change by peak, you can temporarily filter out the new in-silico peptide in the Peptides table by setting the Validation to “Uncertain”, refilter, and then update the Dose Response Fold Change report tab. This will produce Fold Change data for just the original peptide which can subsequently be filtered out to only show Fold Change data for the in-silico peptide by temporarily filtering out the original peptide using the same process.

Filtering data

If you wish to exclude an exposure from the analysis, please deselect the sample in the Projects table:





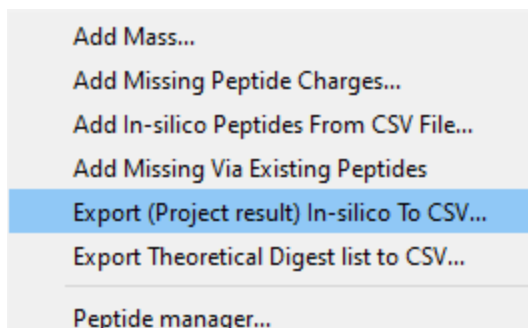
Please DO NOT use the “Exposure Time” field in the Dose Response Fold Change report to exclude an exposure time point from the regression analysis. The “Exposure Time” field should never be used for any filtering. In addition, please do not change the layout of the report in terms of how the column fields are configured.

To export the Dose Response Fold Change data for further analysis, it is helpful to create two CSV files, one for **Free** data and one for **Complex** data. To filter for **Free** sample data, open the “Sample” field in the column to the left of the report and deselect **Complex** in the second type of filter (Show/Hide):

The pivot table report will now show data for **Free** only and this dataset can be exported to a CSV file > “Export pivot table content (CSV)”. Likewise, a CSV file can be created for **Complex** sample data and the two datasets can be joined in an Excel workbook. Please contact the Ralston footprinting group for further help with data presentation.

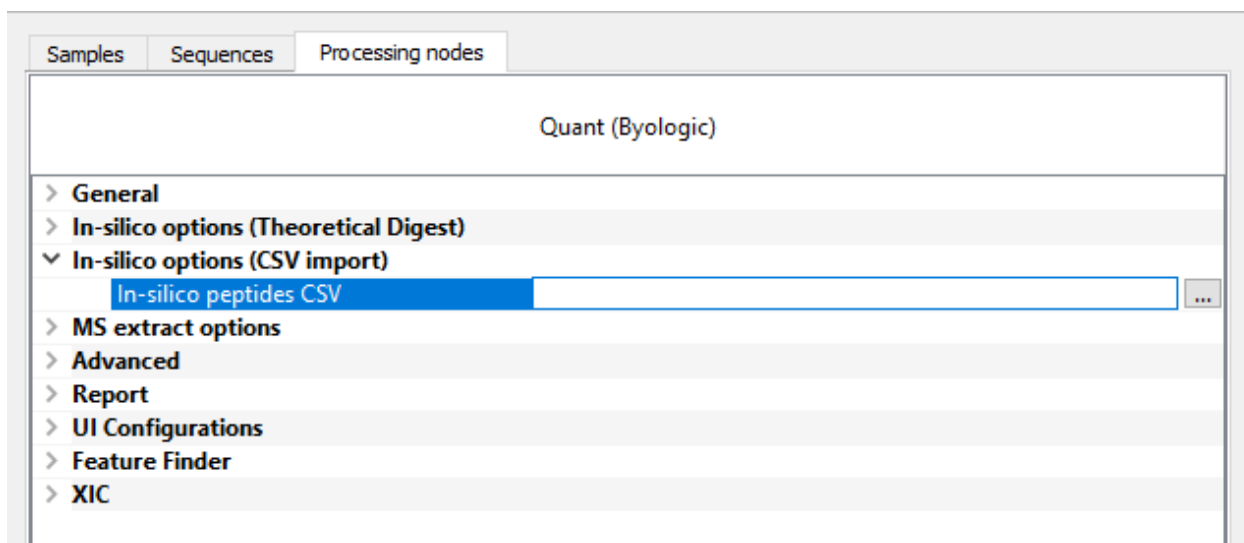
Experiments with more than two conditions

You can take advantage of an already validated project if you have additional conditions to analyze against the free or WT protein. First you will need to create a CSV file based on the project that used MS2 data. The CSV is created by selecting Edit > In-silico Peptides > Export (Project result) In-silico To CSV:



In the D:/**PMI XFMS templates** folder on the PMI server you will find a **Pure in-silico_XFMS** workflow. This is the workflow you will want to use to transfer your completed validation to a new project, assuming that you do not need to perform an MS2 search. In the **Pure in-silico_XFMS** workflow, drag your sample files into the Samples tab and then fill out the Sample and Exposure Time columns as described above. In the Sequences tab, browse for your FASTA sequence file – you might have to expand the file format types in File Explorer to “All” if the FASTA is in txt format.

In the Processing nodes tab, the only thing you have to do is to point to your CSV file location by clicking the three dots at the far right under CSV import:



Check the in-silico project to make sure the CSV was applied correctly. In the PTM Summary Tab, the overall sequence coverage should look as you expect from the original project. You will most likely have to finetune the peptide “XIC Start” and “XIC End” times as there might be slight sample-to-sample RT shifts. Enabling “Stacked Plots” is helpful in checking and setting XIC integration boundaries.