KIT QUANTA - standardization kit for absolute protein quantitation: study of the digestion kinetic of the chimeric protein standard

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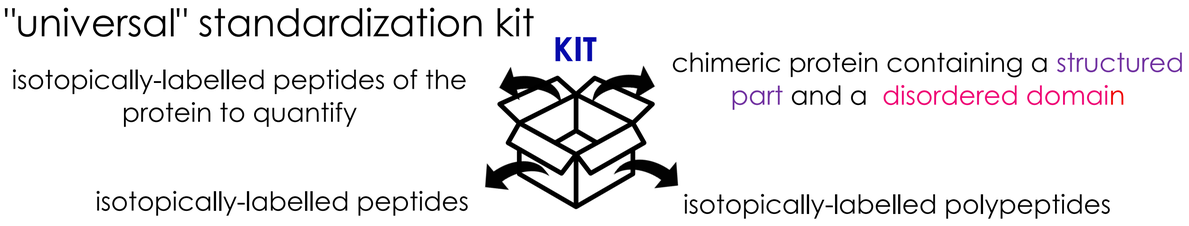
Framework

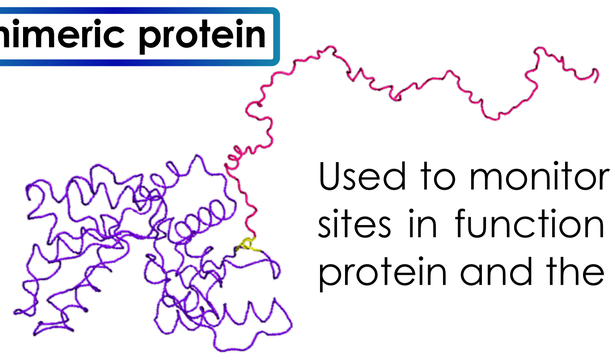
In the world of biomarker discovery, up to now, only a few biomarkers have been validated. The **validation** remains a crucial step in the development of a biomarker but is quite **challenging** and **lengthy**. In this context, the **development of high-throughput techniques** is therefore needed. Mass spectrometry based methods such as **Selected Reaction Monitoring** (SRM) has become a prominent choice for large-scale protein quantification because of its **multiplexing capability** and **specificity**. The difficulty of this approach lies in the implementation of **standardization** for absolute quantification. Different standardization approaches now exist in proteomics but all have their limitation. One example is the use of isotopically-labelled peptides for the absolute quantification of proteins. This method is highly specific of the protein to quantify but does not take into account all the **bias** introduced at each step of the process applied before SRM analyses.

Our solution

Development of a comprehensive standardization strategy aiming to control the entire sample preparation process thanks to a "universal" standardization kit

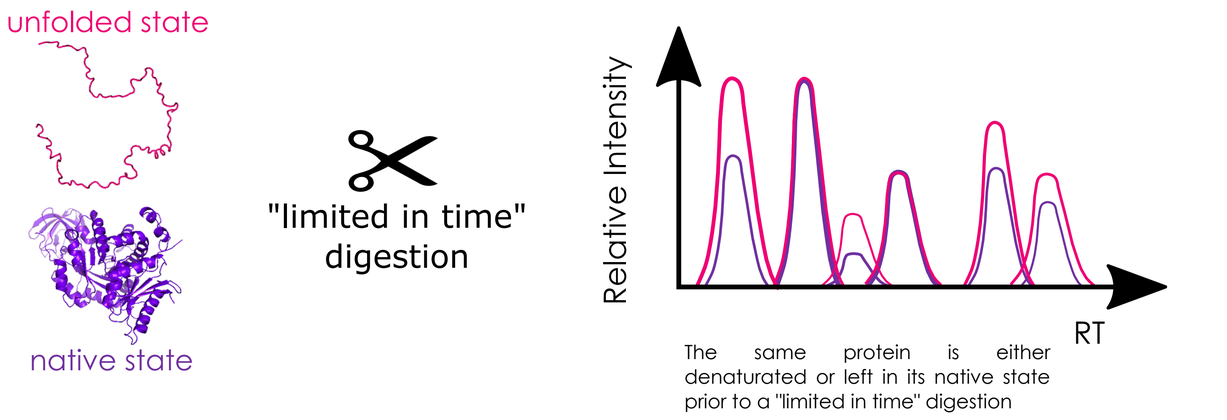


Chimeric protein



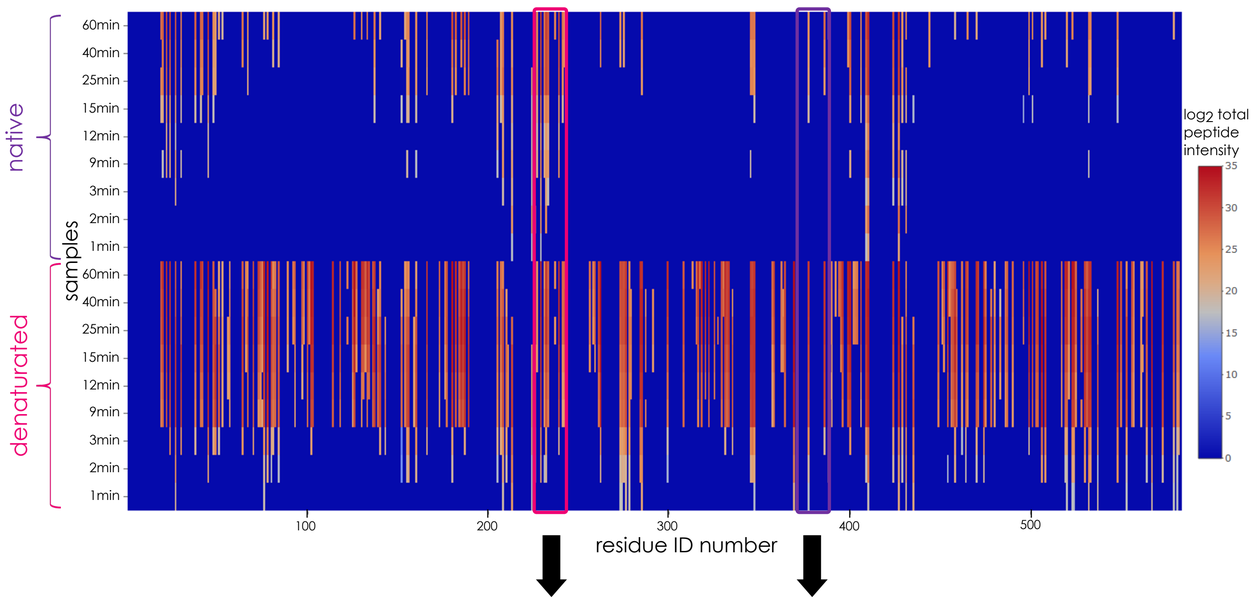
Used to monitor the accessibility of cleavage sites in function of the structural state of the protein and the digestion protocol applied

Methodology



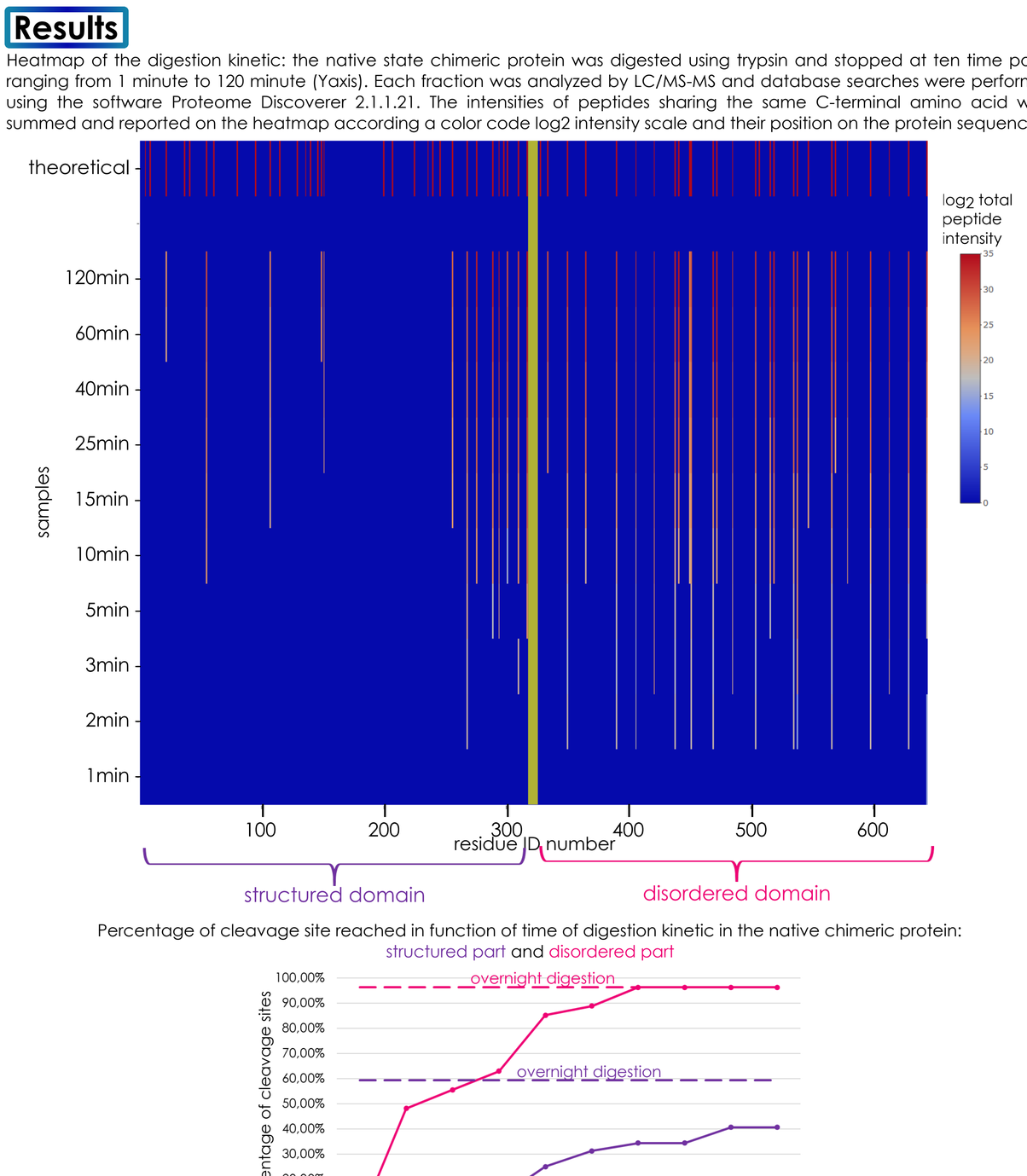
Proof of concept

Heatmap of the digestion kinetic: the BSA was either denaturated by reduction and alkylation of the disulfide bridges and heated or left in a native state prior applying a multi-enzymatic limited digestion (MELD) approach. Nine digestion time points were performed ranging from 1 minute to 60 minutes (Yaxis). Each fraction was analyzed by LC/MS-MS and database searches were performed using the software Proteome Discoverer 2.1.1.21. The intensities of peptides sharing the same C-terminal amino acid were summed and reported on the heatmap according a color code log2 intensity scale and their position on the protein sequence. By comparing the digestion kinetic of the native and denatured protein, protein zones more resistant to enzymatic digestion can be highlighted and correlated with the 3D structure of the protein.



Results

Heatmap of the digestion kinetic: the native state chimeric protein was digested using trypsin and stopped at ten time points ranging from 1 minute to 120 minute (Yaxis). Each fraction was analyzed by LC/MS-MS and database searches were performed using the software Proteome Discoverer 2.1.1.21. The intensities of peptides sharing the same C-terminal amino acid were summed and reported on the heatmap according a color code log2 intensity scale and their position on the protein sequence.



Percentage of cleavage site reached in function of time of digestion kinetic in the native chimeric protein: structured part and disordered part

Conclusions and future steps

Peptides from the expected disordered domain are released more quickly than peptides from the structured part. At the end of the kinetic, the number of peptides as well as their intensity is greater in the disordered domain. The difference in accessibility of cleavage sites depending on the conformational environment of the protein is then proved. As future works, different protocols will also be experiment to evaluate the performance of digestion of both domains under denaturing conditions.

References

Schopper *et al*, *Nature Protocol*, vol. 12, no. 11, pp. 2391-2410, 2017

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Acknowledgments

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