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A protein profiling strategy for Periodontal Disease applications: the Perio-SalivaPRINT

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Background

- Several clinical situations have characteristic molecular dysregulations.

- Strategies are needed to identify the phenotypes characteristic of these dysregulations.

- Some molecular data underlying these deregulations can be found in saliva and have been annotated in databases (SalivaTecDB).

- Our group has developed a strategy that allows the establishment of saliva protein profiles reflecting different conditions (health and disease) that can be integrated with clinical data (SalivaPRINT Toolkit).

- **OBJECTIVE:** the present work aims to identify such Periodontal Diseases (PD)-specific protein profiles.

Analysis Pipeline

01 Saliva Colection
Unstimulated whole saliva collected from a group of healthy subjects and a group of PD patients.

02 Capillary electrophoresis
Salivary proteins separated by the Experion™ automated capillary electrophoresis.

03 SalivaPrint Toolkit
Protein profiles of each condition analyzed by SalivaPrint Toolkit in order to identify major differences in the profiles.

04 SalivaTecDB
Protein profiles of each condition integrated with protein data retrieved from our in-house database (SalivaTecDB).

05 Functional analysis
Functional analysis of the protein data to clarify dysregulated mechanisms of the disease or propose biomarker panels.

Figure 1 – Schematic representation of the proposed Analysis Pipeline.
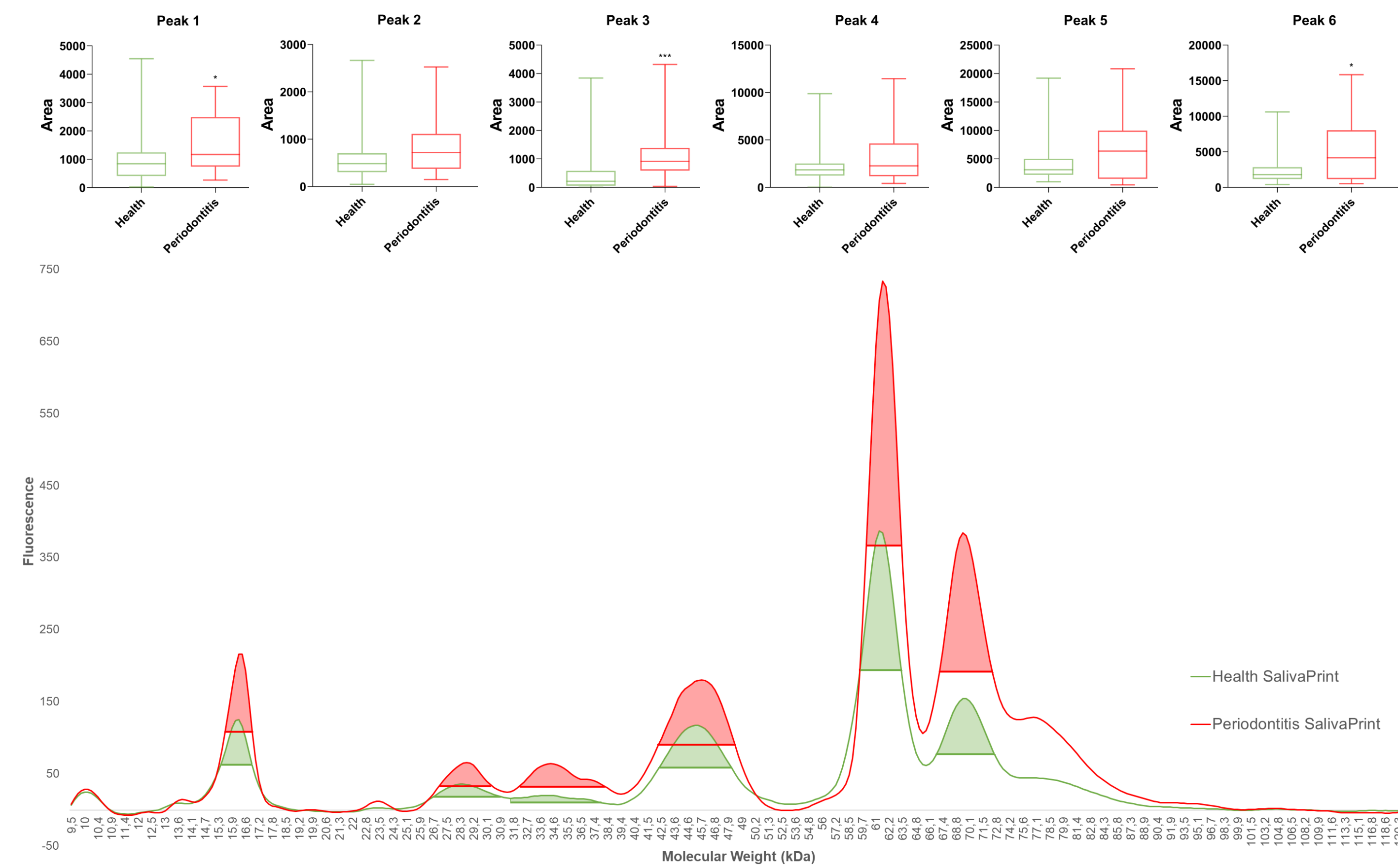
Preliminary Results

Figure 2 – Average SalivaPrint for Periodontitis *vs.* Health. Statistically significant differences were detected in peaks 1, 3 and 6 ($p \leq 0.05$).

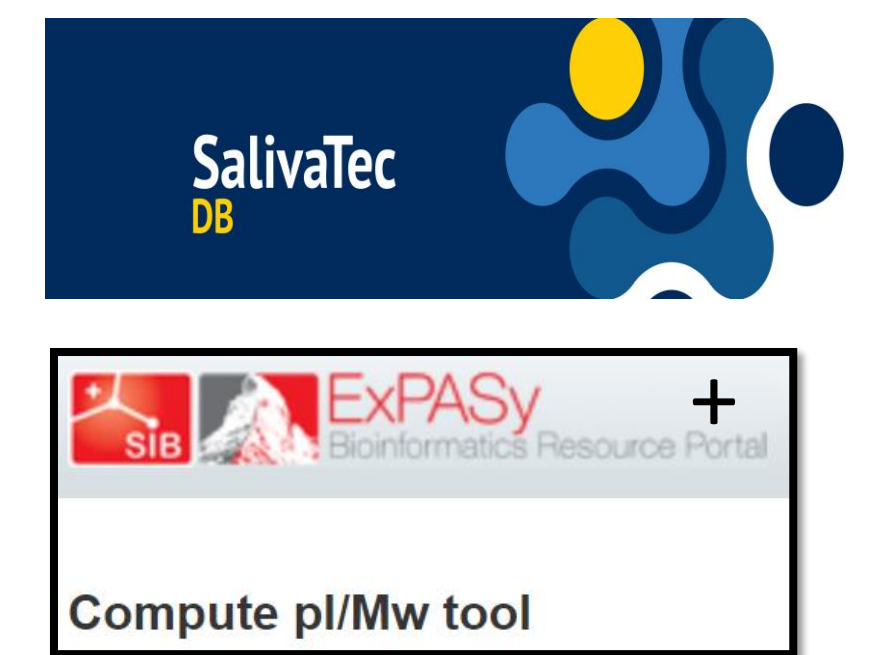|	Periodontitis		
	Min MW	Max MW	Top MW
Peak 1	15.6	16.9	16.4
Peak 2	27.1	30.3	28.8
Peak 3	32.3	38.2	34.3
Peak 4	42.5	48.2	45.7
Peak 5	60.3	63.2	61.6
Peak 6	67.4	72.2	64.4

Table1 – Molecular weight ranges of each of the peaks of the Periodontitis protein profiles. Significantly different peaks are indicated in red ($p \leq 0.05$).

SalivaTec DB

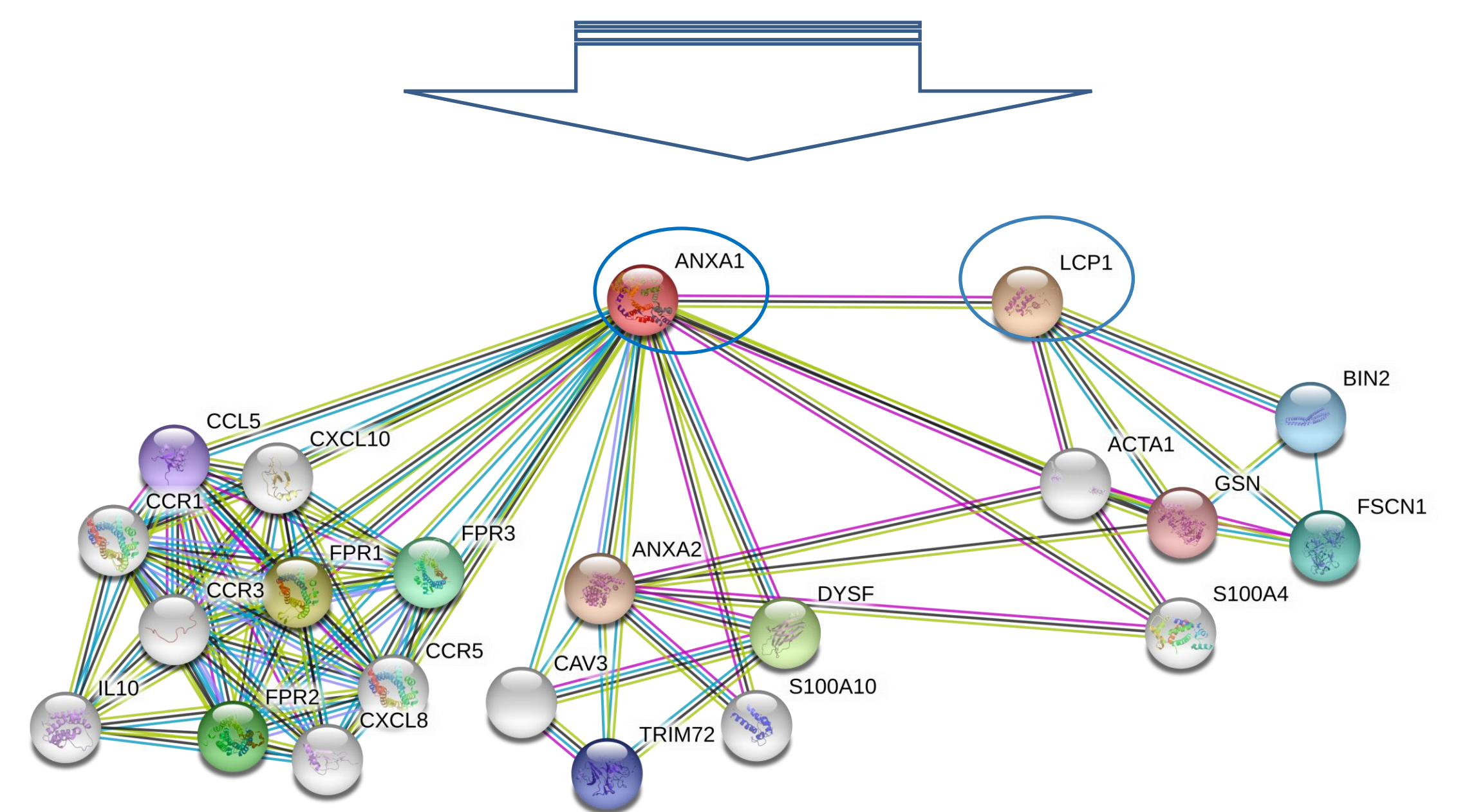ExPASy Bioinformatics Resource Portal
Compute pI/Mw tool

Figure 3 – Two of the Periodontal Diseases Biomarkers suggested by Rosa *et. al.* (2014) can be found in Peak 3 (Annexin A1: ANXA1) and Peak 6 (Plastin-2: LCP1). These Biomarkers reflect a decreased antimicrobial defense (Annexin A1) and Th cell migration/activation (Plastin-2).
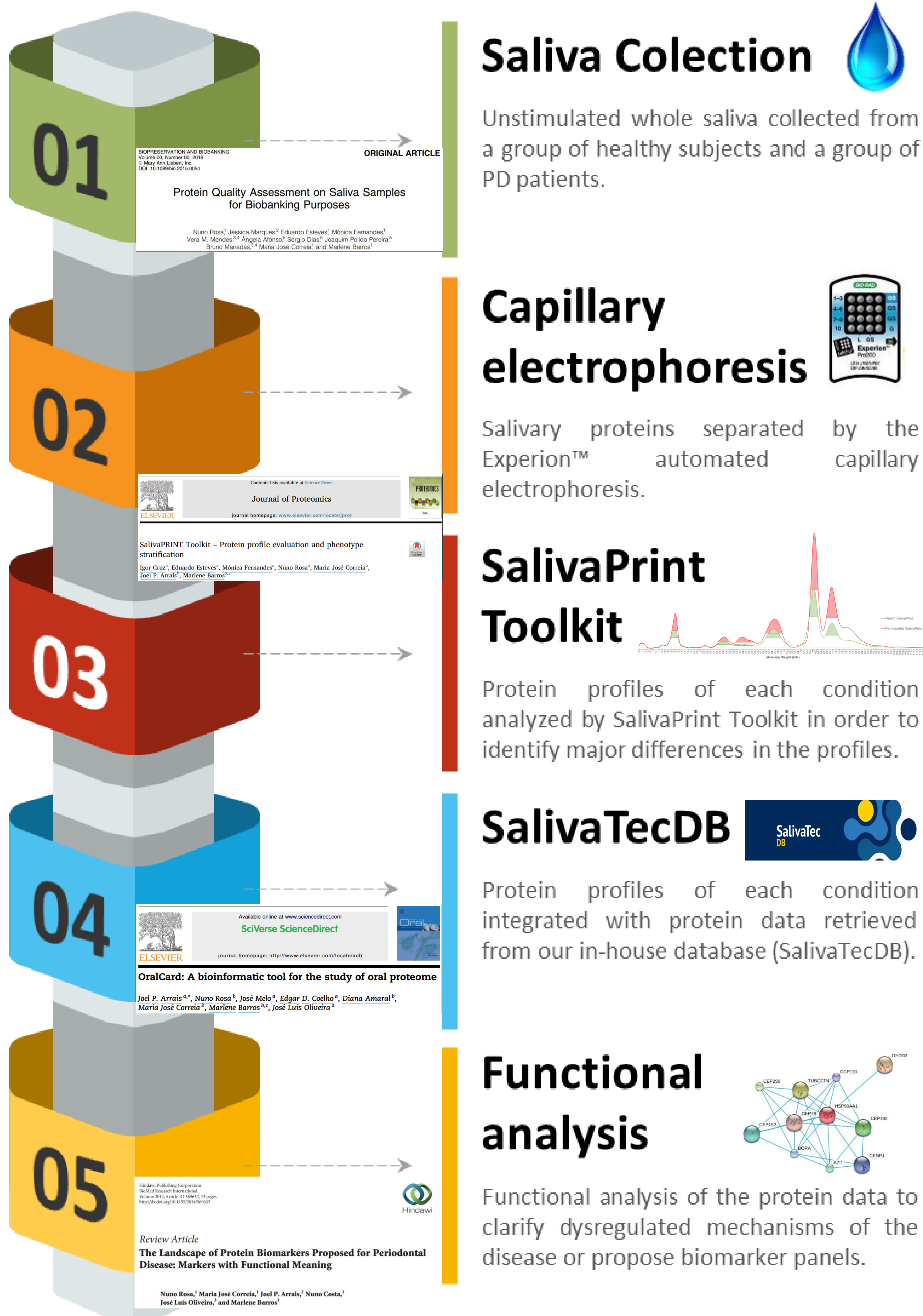
Future Perspectives

- The strategy used enables the determination of a total protein profile from saliva characteristic of each Periodontal Disease status - the Perio-SalivaPrint.

- The use of the SalivaPrint Toolkit allows the identification of molecular weight ranges altered in PD.

- Using SalivaTecDB we can suggest proteins potentially involved in the underlying dysregulated mechanisms of the disease.

References

1. Rosa N, Marques J, Esteves E, Fernandes M, Mendes VM, Afonso Â, et al. Protein Quality Assessment on Saliva Samples for Biobanking Purposes. Biopreservation Biobanking. Agosto de 2016;14(4):289–97.
2. Rosa N, Correia MJ, Arrais JP, Costa N, Oliveira JL, Barros M. The landscape of protein biomarkers proposed for periodontal disease: Markers with functional meaning. BioMed Res Int. 2014;2014.
3. Cruz I, Esteves E, Fernandes M, Rosa N, Correia MJ, Arrais JP, et al. SalivaPRINT Toolkit – Protein profile evaluation and phenotype stratification. J Proteomics. 16 de Janeiro de 2018;171:81–6.
4. Arrais JP, Rosa N, Melo J, Coelho ED, Amaral D, Correia MJ, et al. OralCard: A bioinformatic tool for the study of oral proteome. Arch Oral Biol. 2013;58(7):762–72.
5. Rosa N, Correia MJ, Arrais JP, Lopes P, Melo J, Oliveira JL, et al. From the salivary proteome to the OralOme: Comprehensive molecular oral biology. Arch Oral Biol. 2012;57(7):853–64.
6. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 8 de Janeiro de 2019;47(D1):D607–13.