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TECHNICAL CONSIDERATIONS OF TEAR PROTEOMICS ANALYSIS USING SCHIRMER STRIPS AND TIMSTOF PRO

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1. INTRODUCTION

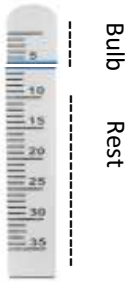
Tear Fluid

- Responsible for <u>lubrication</u> and <u>protection</u> of ocular surface (OS) & <u>optical properties</u> of the eye[1]
- Useful tool for the evaluation of health and disease states of OS [1]
- Valuable source for biomarkers and new diagnostic procedures [1 2]

Which collection method?

<u>Schirmer strip (ScS)</u>

- Standard clinical test for tear production, evaluation & tear collection [3]
- Convenient, rapid, reliable [4]
- Collects both **tear fluid** and **conjunctival cell**s [5].
- More proteins are collected to evaluate OS diseases [6].



Which tool for tear proteomics?

<u>Mass Spectrometry - timsTOF Pro*</u>

- Highly efficient and sensitive tool for tear proteome analysis [7 8]
- Determines changes of protein quantity[7].
- Adds **ion mobility** as a third dimension of separation after nano-LC** and m/z*** [8].

** timsTOF Pro: Trapped ion mobility spectrometry coupled with quadrupole time-of-flight mass spectrometry*
*** nano-LC: Nalo- liquid chromatography*
**** m/z: Mass to charce ratio*

<u>OBJECTIVE</u>

Investigation of healthy human tear proteins extracted from different parts of the Schirmer strips (whole strips (W), bulbs (B) and rest of the strips (R))using timsTOF Pro.

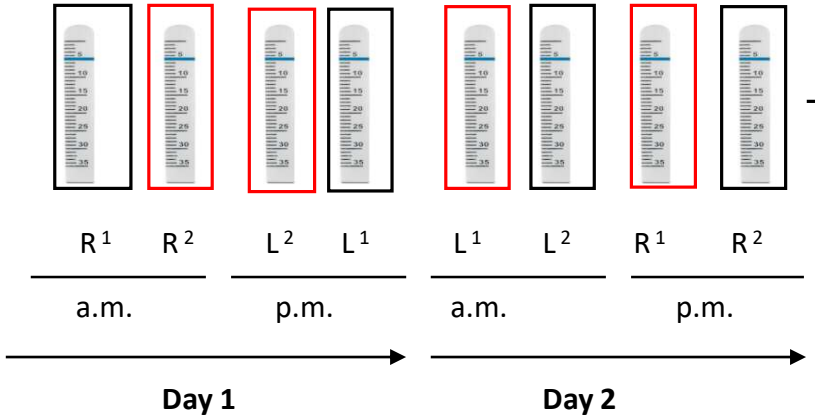
2. METHODS

A. Tear sample collection and processing

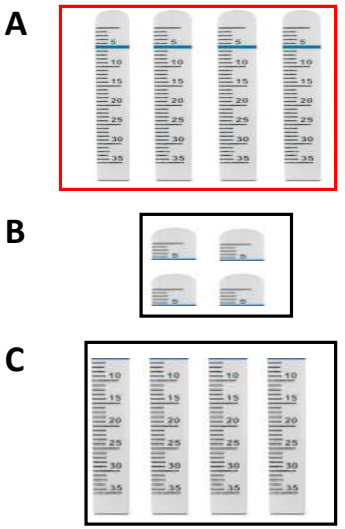
Schirmer strip (ScS) test

2 healthy subjects

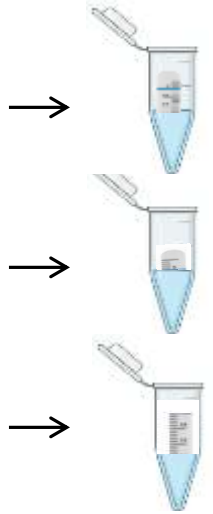
Collection of the ScS



Pooling of ScS



Protein extraction



R, right eye; L, left eye; a.m., in the morning; p.m., in the afternoon; [1], healthy subject-1; [2], healthy subject-2

B. Steps to protein identification

1. Sample preparation

- **Protein quantification**
- **Protein normalization**
- **Protein reduction, digestion and alkylation**

2 . Nano-LC separation

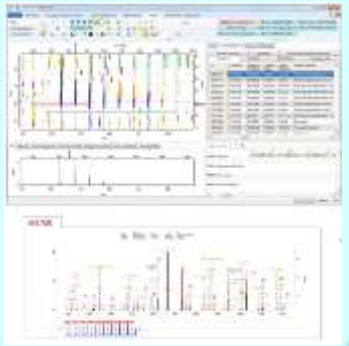
nanoElute UHPLC*

3. MS/MS analysis**

timsTOF Pro®

4. Protein Identification

(MaxQuant)

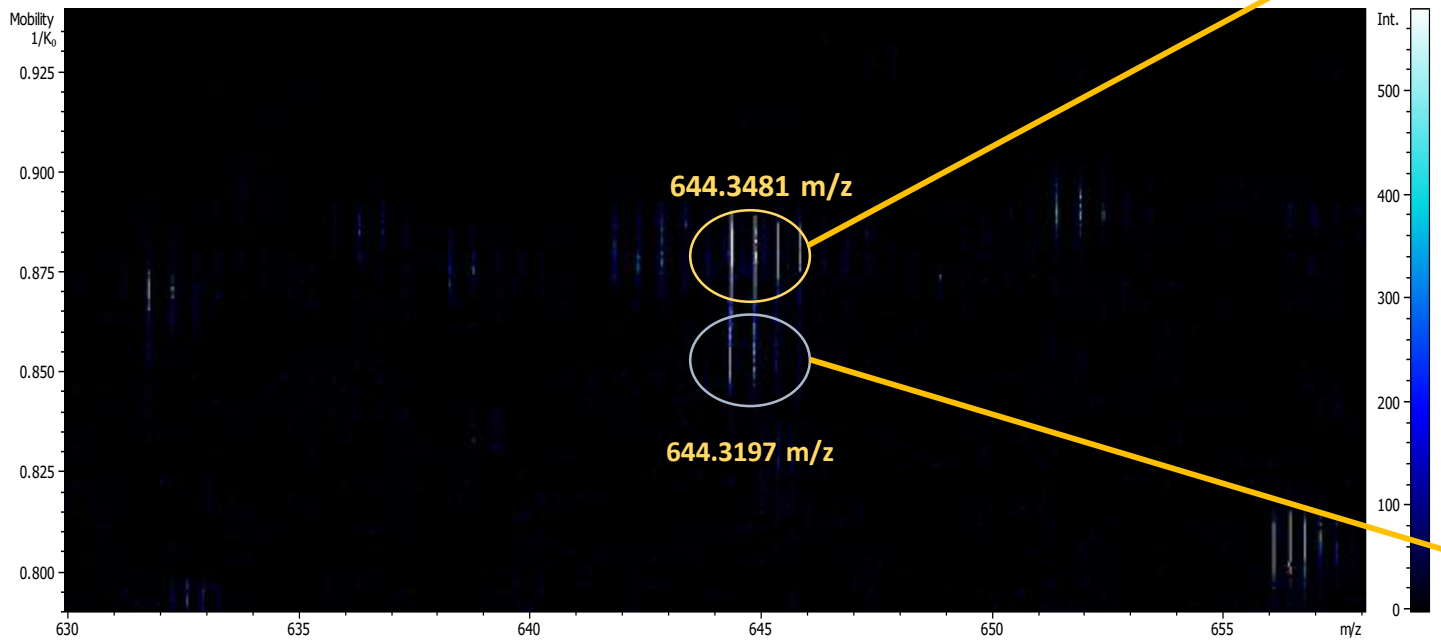


*Ultra High Pressure Liquid Chromatography; ** Tandem mass spectrometry

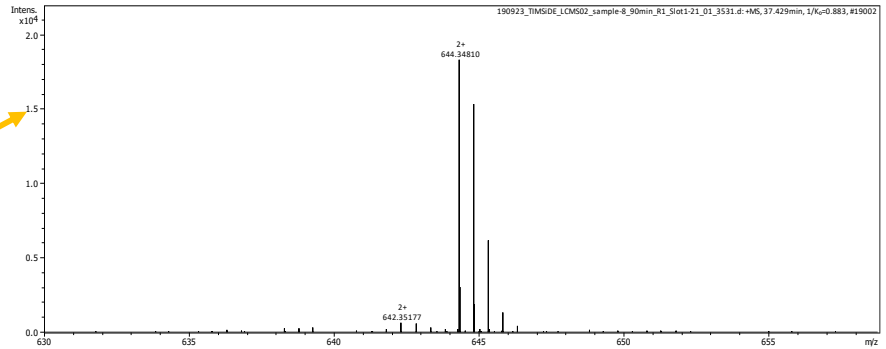
3. RESULTS (1)

Separation of different proteins with the same m/z ratio

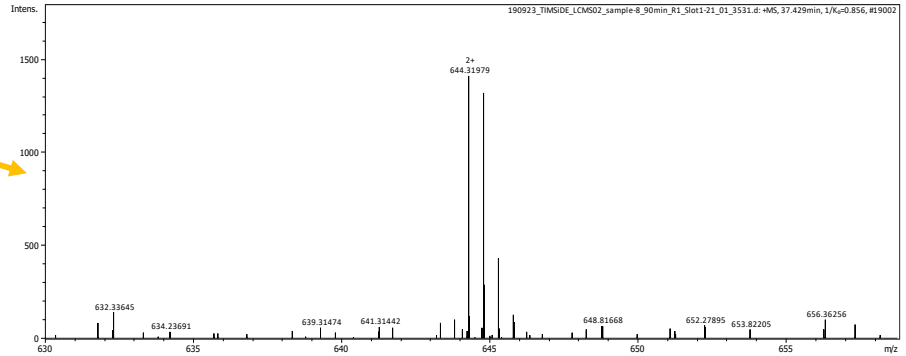
MOBILITY
$(1/k_0)$*



Heat-map visualization of ion mobility



Leukocyte elastase inhibitor
644.3481 m/z
$1/k_0$= 0.883

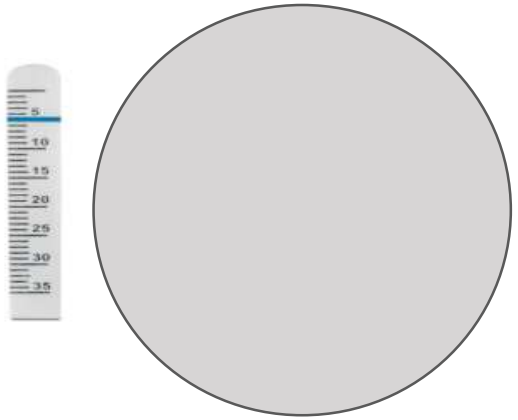


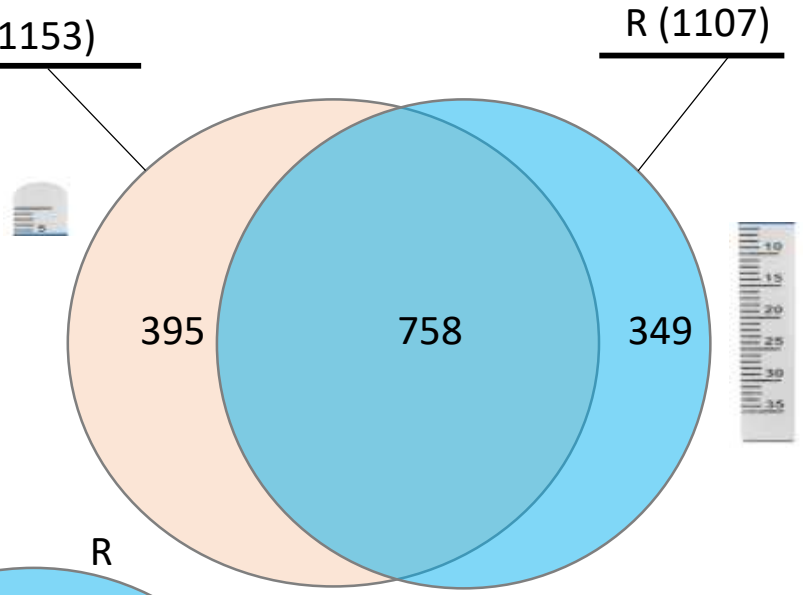
Immunoglobulin κ variable 2-24
644.3197 m/z
$1/k_0$ = 0.856

*$1/k_0$: Drift time

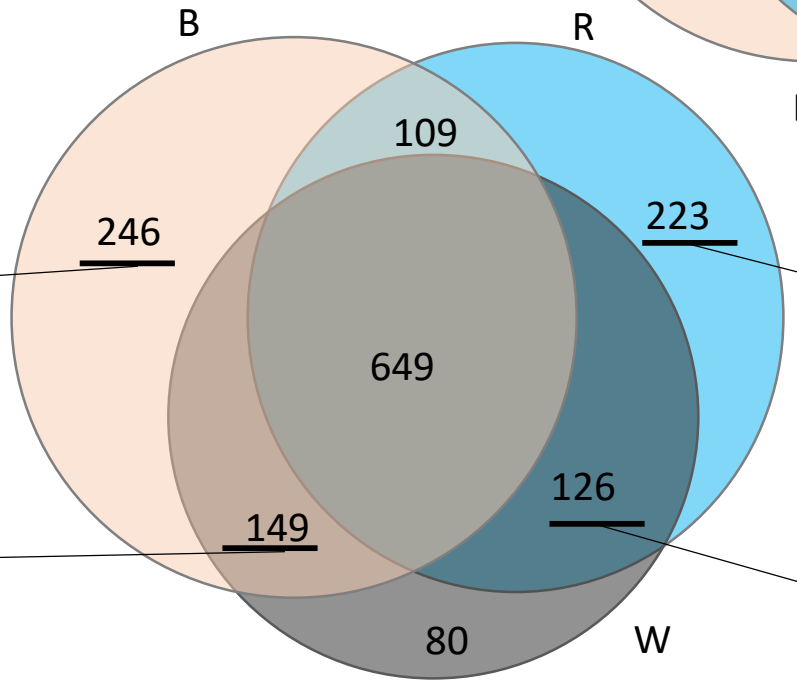
3. RESULTS (2)

The Number of Identified Proteins in the whole strip (W), the bulb (B) and rest of the strip (R)





B+R = **1502 (49.6%↑)**



B+R+W = **1582**

Unique proteins in B
MUC1 and MUC4
Annexin A6
Complement C6, C1QB
Dihydropteridine reductase

Only in B&W
MUC5AC
Caspase-3
Superoxide Dismutase-2

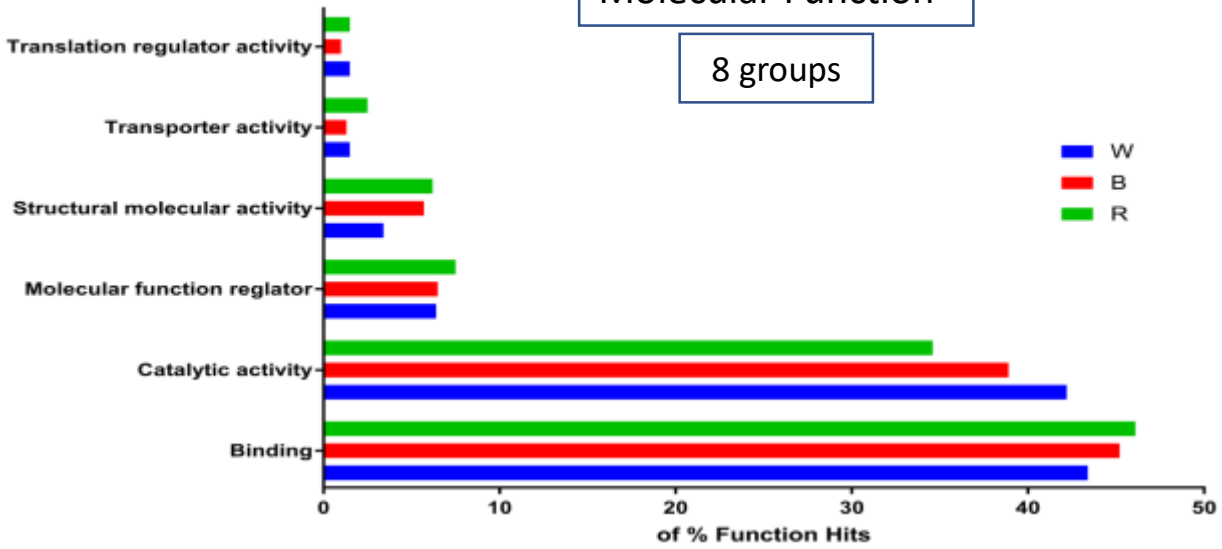
Unique proteins in R
Serpin-B3, -B4, -B7, -B12, -B13
Protein POF1B
Kallikrein-6, -7, -9, -10, -13
Epiplakin
Tight junction protein ZO-2

Only in R&W
Junction plakoglobin
Desmocollin-1
Galectin-7

Gene Ontology Analysis of Identified Proteins

Molecular Function

8 groups

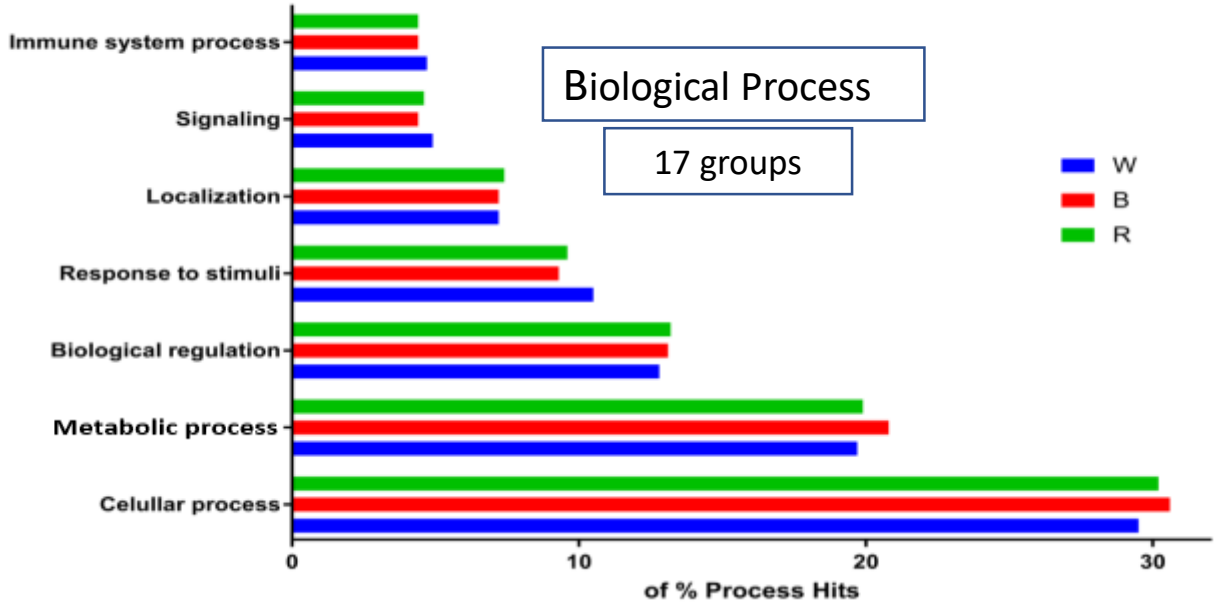


No significant differences among W, B and R

Catalytic activity + Binding = ~80%

Biological Process

17 groups



Cellular process + Metabolic process = ~50%

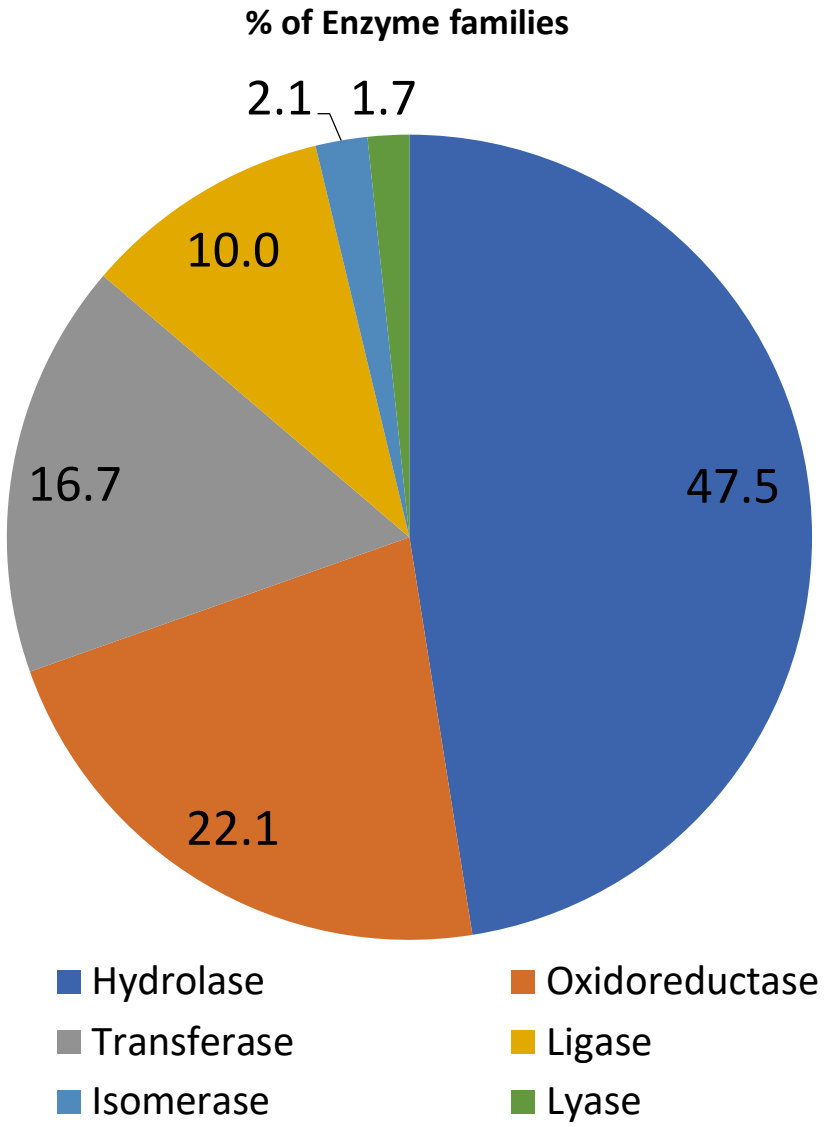
3. RESULTS (4)

Comparison of protein classes* in different parts of the strip

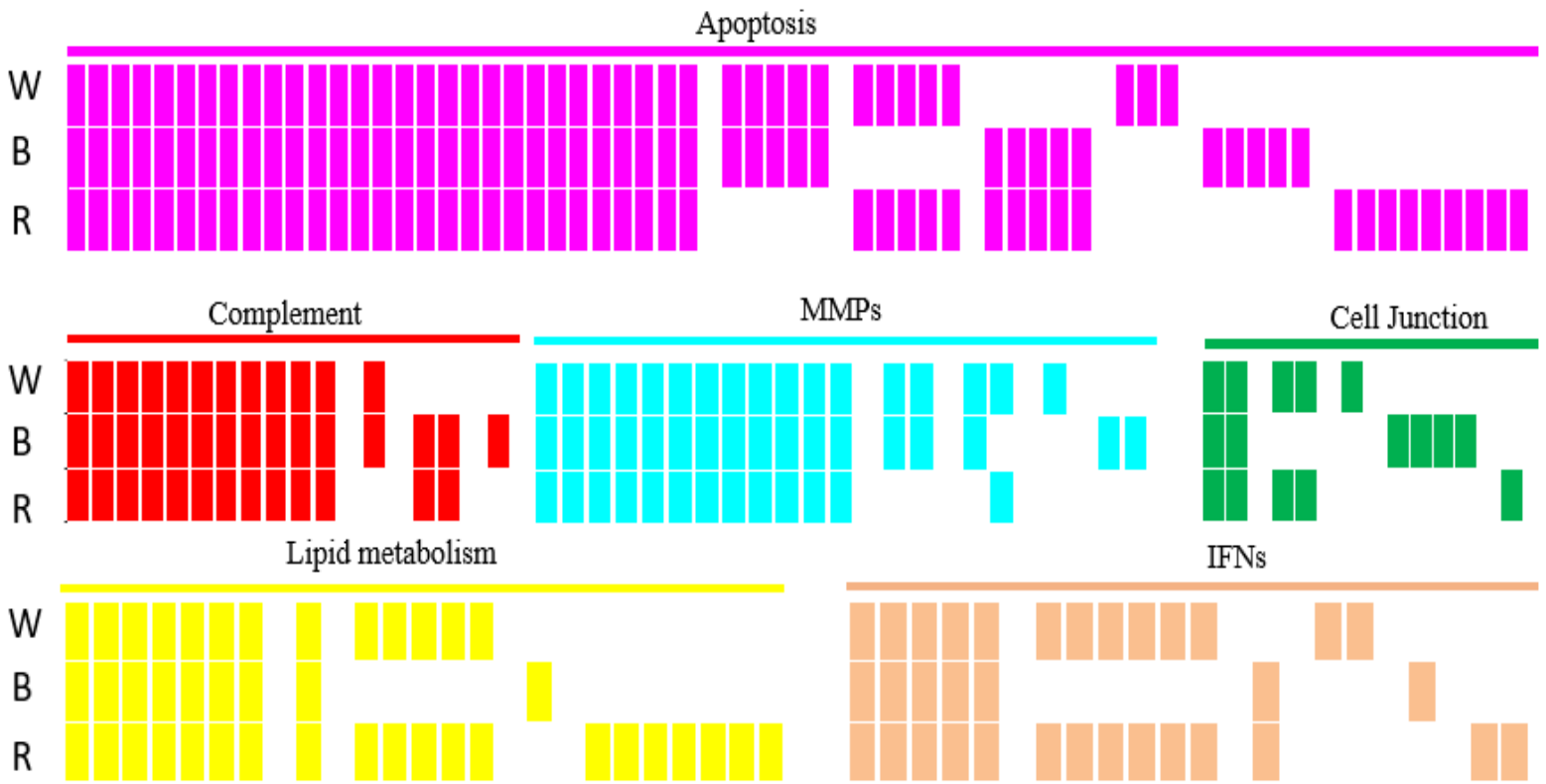
Protein Class	W	B	R
metabolite interconversion enzyme	188	224	177
protein modifying enzyme	112	102	105
cytoskeletal protein	72	76	79
defense/immunity protein	66	63	63
protein-binding activity modulator	57	67	64
translational protein	53	83	84
calcium-binding protein	30	32	30
chaperone	28	28	31
membrane traffic protein	25	25	29
extracellular matrix protein	19	13	16
scaffold/adaptor protein	19	26	20
nucleic acid metabolism protein	17	29	42
transfer/carrier protein	17	17	19
transporter	16	15	24
transmembrane signal receptor	12	10	10
intercellular signal molecule	11	12	15
cell adhesion molecule	8	8	9
chromatin/chromatin-binding	8	15	13
gene-specific transcriptional regulator	5	6	6
structural protein	3	2	3
viral or transposable element protein	0	0	1
cell junction protein	0	1	1

*Protein classes were analyzed using **Panther** software.

480 enzymes identified from W+B+R



Proteins involved in various signalling pathways



Each bar represents one protein

In Apoptosis → 61

Complement cascade → 15

Matrix metallopeptidases (MMPs) → 18

Cell Junction → 10

Lipid Metabolism → 21

Interferons (IFNs) → 17

proteins from the whole strip (W), the bulb (B) and rest of the strip (R) were involved.

4. CONCLUSIONS

- ✓ **Processing separately the two parts of the Schirmer strip increases the number of identified proteins dramatically compared to processing the entire strip.**

- ✓ **Enzymes constitute the largest group in tear proteome.**

- ✓ **The created dataset can help to model and compare multiple signalling pathways associated with Dry Eye Disease (DED) pathophysiology.**

- ✓ **TimsTOF Pro could bring a new dimension to protein profiling in DED thanks to its unique sensitivity that enables deep proteomics analysis from a limited sample.**

5. REFERENCES

1. Azkargorta, M., Soria, J., Acera, A., Iloro, I. & Elortza, F. Human tear proteomics and peptidomics in ophthalmology: Toward the translation of proteomic biomarkers into clinical practice. *J. Proteomics* **150**, 359–367 (2017).
2. von Thun und Hohenstein-Blaul, N., Funke, S. & Grus, F. H. Tears as a source of biomarkers for ocular and systemic diseases. *Exp. Eye Res.* **117**, 126–137 (2013).
3. Quah, J. H. M., Tong, L. & Barbier, S. Patient acceptability of tear collection in the primary healthcare setting. *Optom. Vis. Sci.* **91**, 452–458 (2014).
4. Posa, A. *et al.* Schirmer strip vs. capillary tube method: Non-invasive methods of obtaining proteins from tear fluid. *Ann. Anat. - Anat. Anzeiger* **195**, 137–142 (2013).
5. Green-Church, K. B., Nichols, K. K., Kleinholz, N. M., Zhang, L. & Nichols, J. J. Investigation of the human tear film proteome using multiple proteomic approaches. *Mol. Vis.* **14**, 456–70 (2008).
6. Nättinen, J., Aapola, U., Jylhä, A., Vaajanen, A. & Uusitalo, H. Comparison of Capillary and Schirmer Strip Tear Fluid Sampling Methods Using SWATH-MS Proteomics Approach. *Transl. Vis. Sci. Technol.* **9**, 16 (2020).
7. Yuen, J., Ma, W., Sze, Y. H. O. N., Bian, J. F. & Lam, T. C. Critical role of mass spectrometry proteomics in tear biomarker discovery for multifactorial ocular diseases (Review). (2021) doi:10.3892/ijmm.2021.4916.
8. Meier, F. *et al.* Online parallel accumulation–serial fragmentation (PASEF) with a novel trapped ion mobility mass spectrometer. *Mol. Cell. Proteomics* **17**, 2534–2545 (2018).