DISTRIBUTION OF PROTEINS IN THE DIFFERENT PARTS OF THE SCHIRMER STRIPS

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1. INTRODUCTION

Tear Film

- Protection, lubrication, and nutrition of ocular surface (OS)[1]
- Optically smooth surface for good refraction [2]
- Accessible and useful source for evaluation of OS diseases, inflammation[3], prognosis and diagnostic purposes [1]
- Tear protein profiles have been extensively investigated in multiple proteomic studies [4, 5].

Collection method of choice?

Schirmer strip (ScS)

- A standard clinical test for tear production, evaluation & collection [6, 7]
- Reliable, rapid [8]
- Collects both **tear fluid** and **conjunctival cells** [9] (More proteins to evaluate OS diseases[10])

Proteomics

TimsTOF Pro* mass spectrometry (MS)

- LC-MS/MS can identify and quantify large numbers of tear proteins [10]
- Highly efficient and sensitive tool for tear proteome analysis [11, 12]
- Multidimensional proteomics (nano-LC**+ ion mobility + m/z ***+TOF) [12]

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

OBJECTIVE

To analyze and compare protein composition in different parts of the Schirmer strips by using a comprehensive proteomics approach based on timsTOF Pro, a highly sensitive mass spectrometry technology.

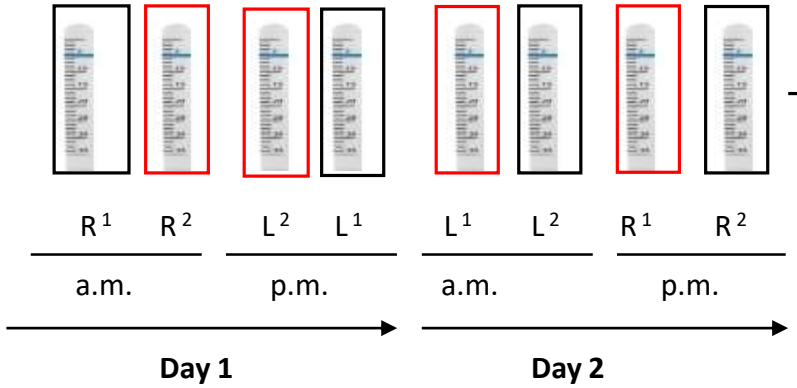
2. METHODS

Tear sample collection and processing

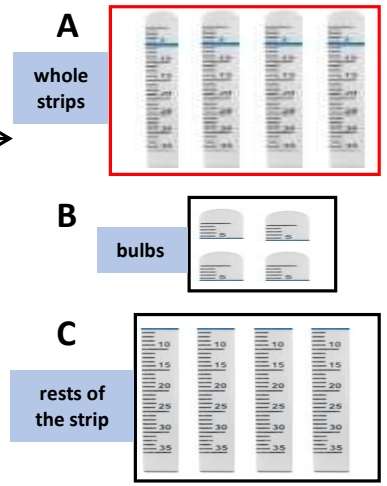
Schirmer tear test (STT)

healthy subjects

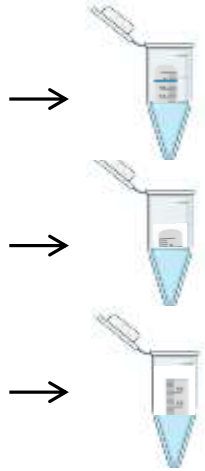
Collection of ScS



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

Steps to protein identification

1. Sample preparation

- **Protein quantification**
- **Protein normalization**
- **Reduction, digestion and alkylation of the proteins**

2 . Nano-LC separation

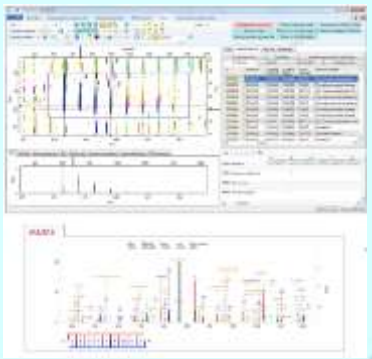
nanoElute UHPLC*

3. MS/MS analysis**

timsTOF Pro®

4. Protein Identification

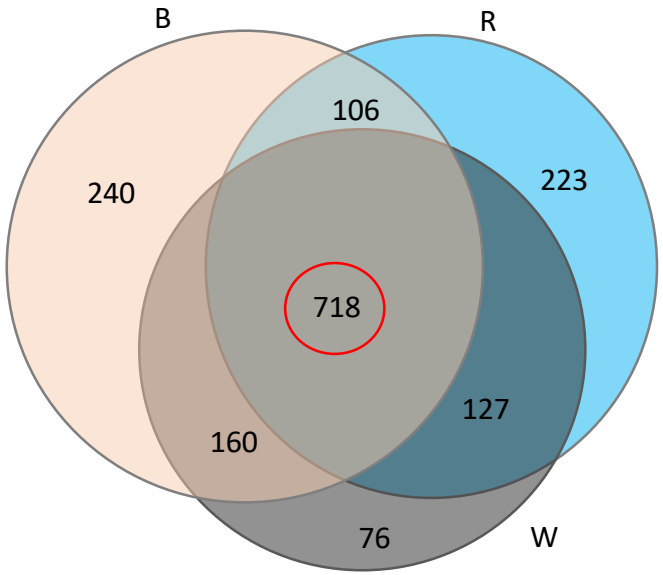
(MaxQuant)



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

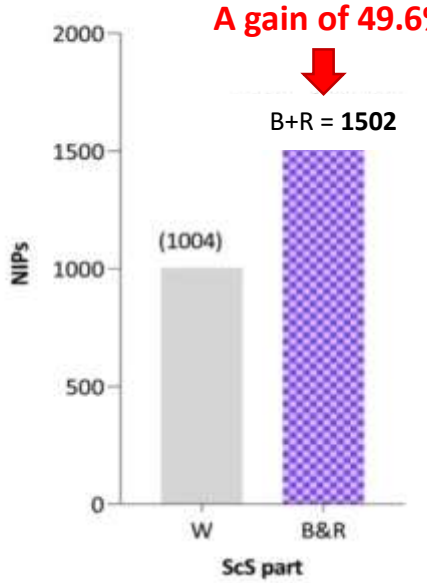
3. RESULTS (1)

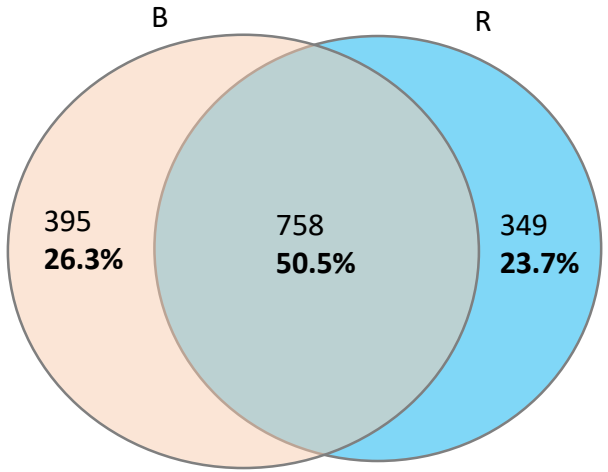
- Number of identified proteins (NIPs)
- W: Whole strip
- B: Bulb
- R: Rest of the strip



B+R+W = **1650**

Elimination of keratins



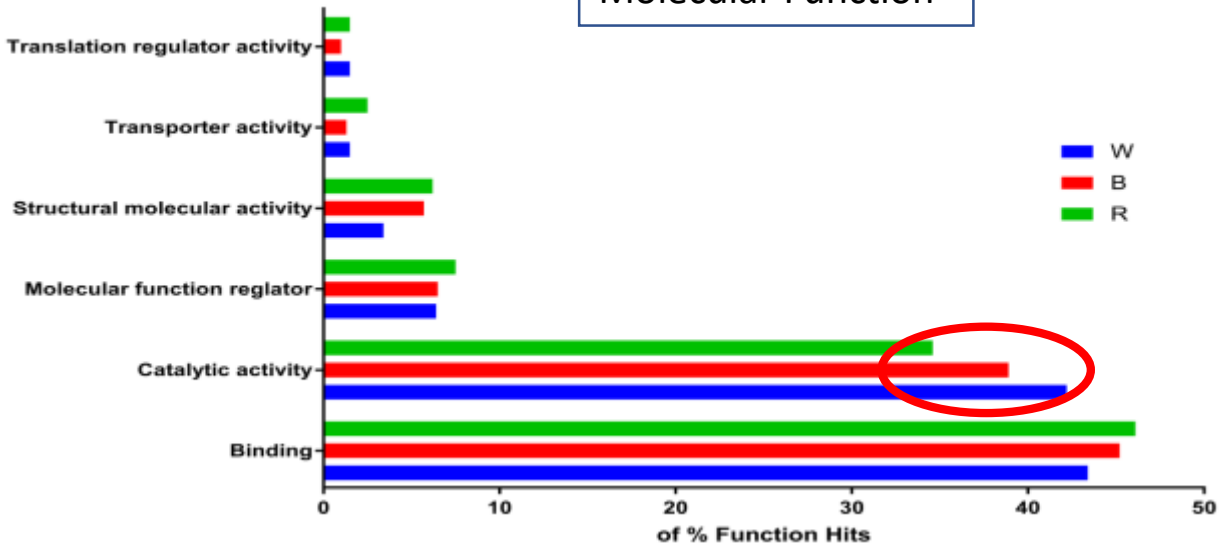


B+R = **1502**

➢ Processing B and R separately, increased the NIPs by 49.6% more than processing the entire strip.

Gene Ontology Analysis of Identified Proteins

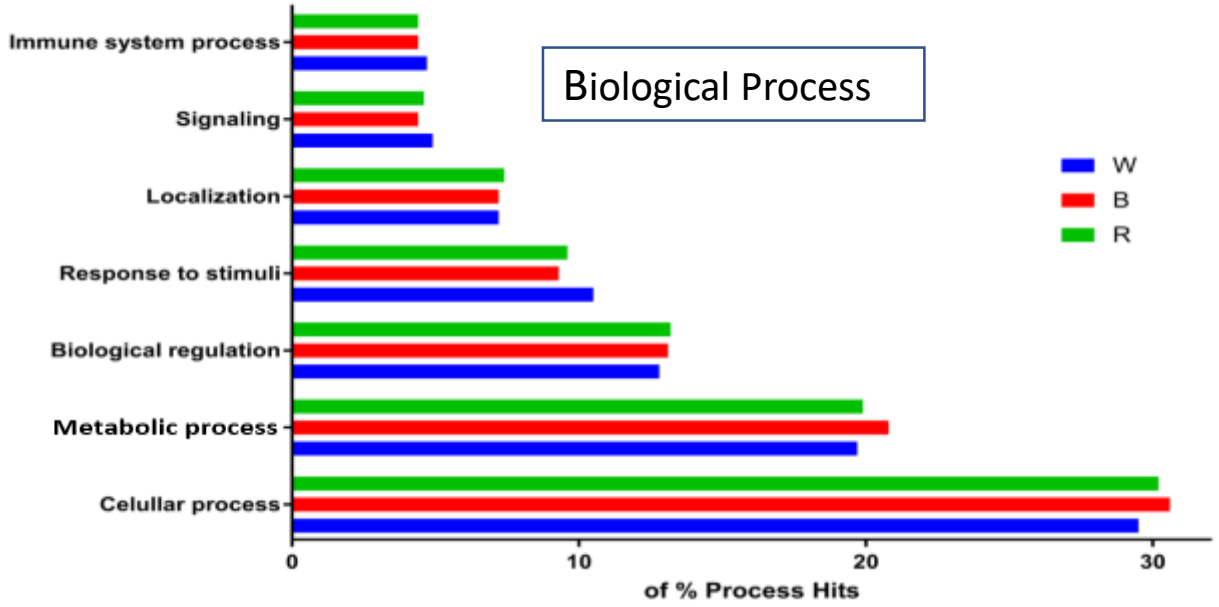
Molecular Function



Except in catalytic activity, no important differences observed in the Gene Ontology analysis of W, B and R

Catalytic activity + Binding = ~80%

Biological Process



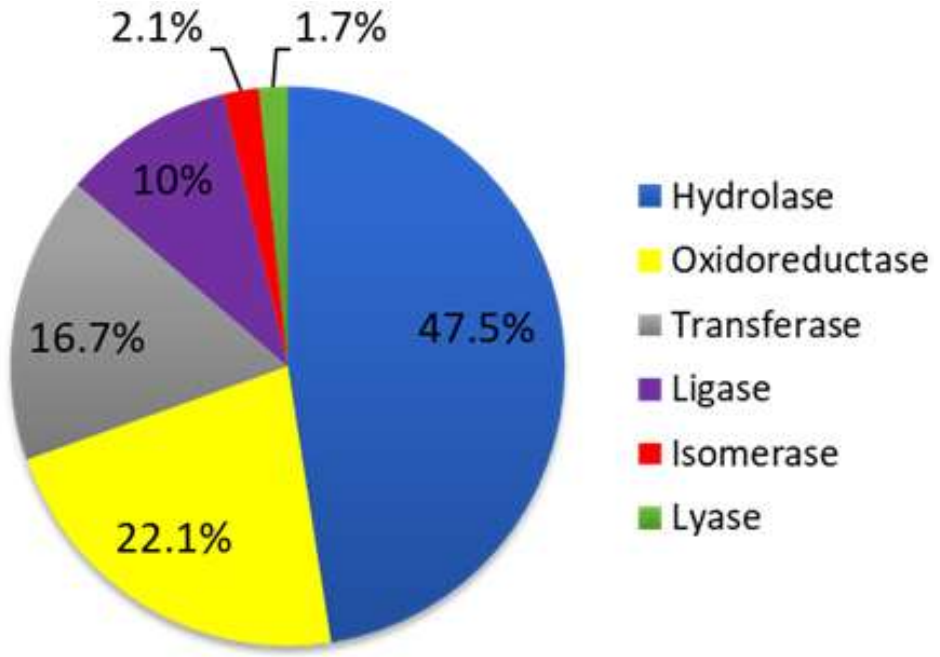
Cellular process + Metabolic process = ~50%

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

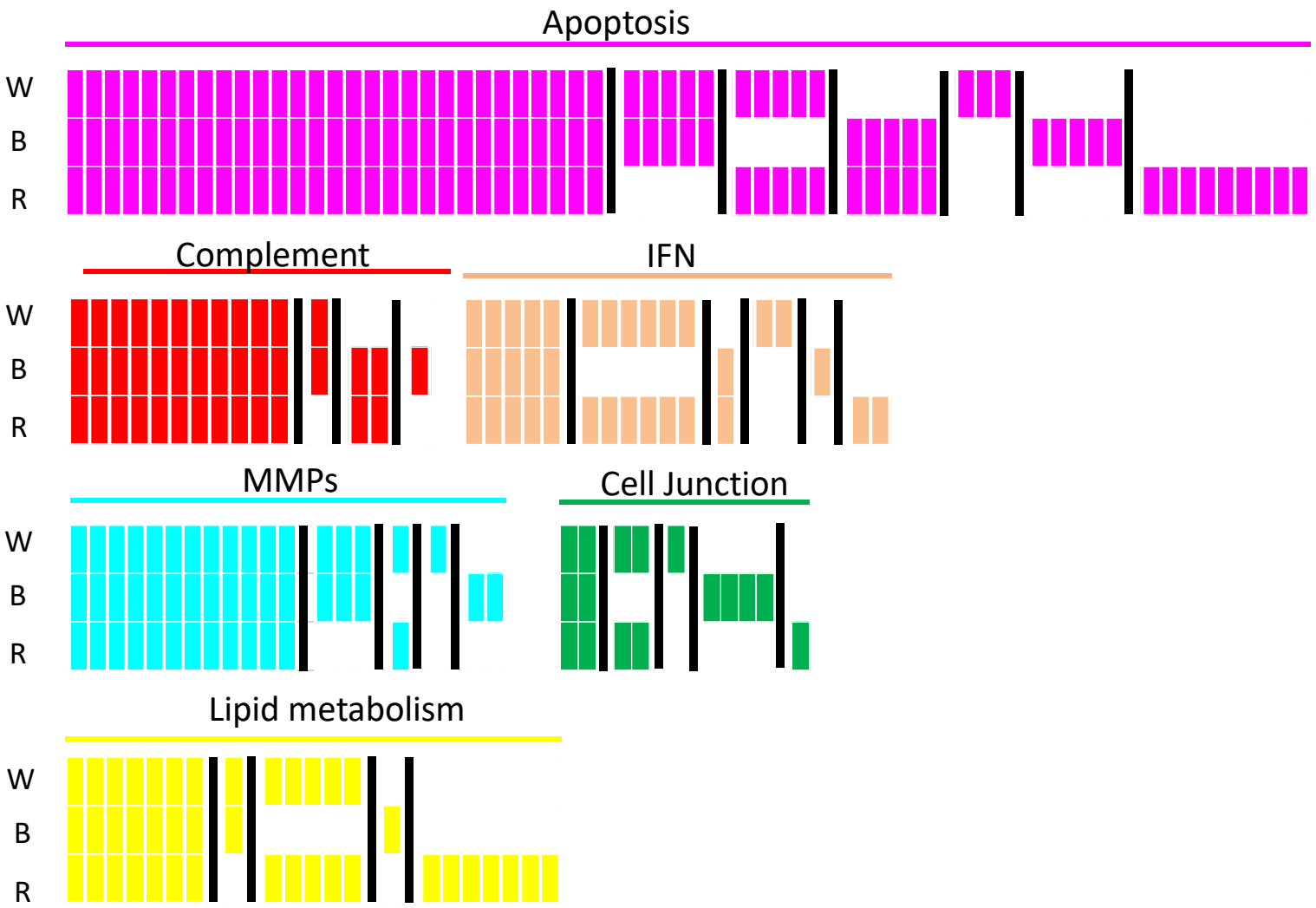
0 10 50 75 125 175 225

Distribution of enzyme families in tear proteome



- **480 enzymes** identified from W+B+R together

Proteins involved in various signaling pathways



The number of total proteins from the whole strip (W), the bulb (B) and rest of the strip (R) involved in these pathways were:

Apoptosis → 61

Complement cascade → 15

Interferon (IFN) → 17

Matrix metalloproteinses (MMPs) → 18

Cell Junction → 10

Lipid Metabolism → 21

Each bar represents one protein except black bars . The black bars to separate the common proteins in different parts of ScS.

4. CONCLUSIONS

- ✓ A total of **1582 proteins** were identified by separately investigating the different parts of the Schirmer strips, with the identification of 49.6% additional proteins.

- ✓ This methodology could improve the pre-analytical steps before MS analysis.

- ✓ Enzymes formed the largest protein group of the tear proteome, with an identification of **480 enzymes** particularly from hydrolase (47.5%) and oxidoreductase (22.1%) enzyme families.

- ✓ The dataset created can help to model and compare multiple signaling pathways associated with ocular surface pathologies.

- ✓ TimsTOF Pro could also add a technical improvement for the investigation of biomarkers in ocular diseases.

5. REFERENCES

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