# TuPA

**Turkish Proteomics Association**

## TuPA International Proteomics Congress, İzmir, Türkiye
## 4th Turkish National Proteomics Congress
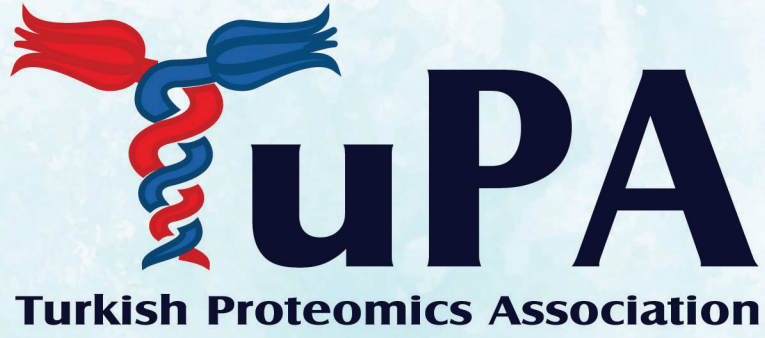
**14-15 October 2022**

**Dokuz Eylül University**

DOKUZ EYLÜL UNIVERSITY
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## ABSTRACT BOOK

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events

Turkish Proteomics Association

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*Scientific Committee is listed alphabetically by last name.*

| October 13th 2022, Thursday | |
|---|---|
| 09:00 | **Registration Desk Opens** |
| 09:30–16:45 | **Course: Targeted Proteomics Analysis** |

| October 14th 2022, Friday | |
|---|---|
| 08:15 | **Registration Desk Opens** |
| 09:00–09:30 | **Opening Speeches**<br>**Prof. Hüray İşlekel**, *Congress President*<br>**Prof. Aysel Özpınar**, *President of Turkish Proteomics Association*<br>**Prof. Nükhet Hotar**, *The Rector of Dokuz Eylül University, Honorary President (If She Deems Appropriate)*<br>***Ensemble Concert,*** *Dokuz Eylül University Conservatory* |
| 09:30–10:15 | **Keynote Speech**<br>**Alexander R. Ivanov, Northeastern University, USA**<br>*Bottom-up, top-down, and native proteomic profiling and glycomic characterization of ng- and sub-ng-level samples, including small cell populations and single cells* |
| 10:15–10:30 | **Break** |
| 10:30–11:30 | **Session 1 - Post-Translational Modification Analysis/New approaches in proteomics**<br>**Chair: Prof. Aysel Özpınar**<br>**Tiziana Bonaldi, European Institute of Oncology, Italy**<br>*Protein post-translational modifications, on histone and beyond, in cancer onset, adaptation and plasticity (ONLINE)*<br>**Michael L. Nielsen, University of Copenhagen, Denmark**<br>*Proteomics-based studies of ADP-ribosylation* |
| 11:30–12:00 | **Break** |
| 12:00–13:00 | **Session 2 - Structural Proteomics**<br>**Chair: Prof. Okay Çağlayan**<br>**Şerife Ayaz Güner, İzmir Institute orf Technology, Türkiye**<br>*In-depth analysis of proteoforms with top-down proteomic approaches*<br>**Hasan Demirci, Koç University, Türkiye**<br>*Bright future of structural biology in Turkey: Structural dynamics of macro molecular complexes* |
| 13:00–14:00 | **Satellite Symposium**<br>**Redokslab/ Thermo Scientific**<br>**M. Tekin Şensoy**<br>*Orbitrap applications in proteomics analysis* |
| 13:00–14:30 | **Lunch Break / Poster Session** |

| | |
|---|---|
| **14:30-16:00** | **Oral Presentations**<br>**Chair: Assoc.Prof. Yasemin Saygıdeğer**<br>**Nazlı Ezgi Özkan Küçük**<br>S01 *Palmitoylation directs PCDH7 to plasma membrane during cell division*<br>**Selahattin Can Özcan**<br>S02 *Nek2 regulates multipolar spindle formation in centrosome amplified cancer cells*<br>**Nazlı Dilara Erdoğdu**<br>S03 *Detection and functional analysis of CHEK2 VUSes in cancer patients*<br>**Burcu Saygıdeğer Demir**<br>S04 *Analysis of the interaction between thymoquinone and oncogenic driver proteins*<br>**Ceyda Seren Ceyhan**<br>S06 *Regulation of keratin 8 phosphoprotome during cancer cell division*<br>**Sinan Kandır**<br>S07 *Equine proteomics: A scoping study of current trends and future aspects* |
| **16:00-16:30** | **Break** |
| **16:30-17:45** | **Session 3 - Multiple Analysis of DNA Repair Proteins**<br>**Chair: TBA**<br>**Miral Dizdaroğlu, National Institute of Standards and Technology (NIST), USA**<br>*Measurement of DNA repair proteins by mass spectrometry (ONLINE)*<br>**Melis Kant, National Institute of Standards and Technology (NIST), USA**<br>*Inhibition of DNA repair proteins in cancer therapy (ONLINE)* |
| **19:00-23:00** | **Congress Dinner** |
| **October 15th 2022, Saturday** |
| **08:45** | **Registration Desk Opens** |
| **09:30-10:30** | **Session 4 - Diseases and Clinical Applications**<br>**Chair: Prof. Hüray İşlekel**<br>**Talat Yalçın, İzmir Institute of Technology, Türkiye**<br>*Proteomic analysis of leishmania parasites isolated from leishmaniasis patients*<br>**Gizem Çalıbaşı Koçal, Dokuz Eylül University, Türkiye**<br>*Protein biomarkers based on liquid biopsy for the diagnosis and prognosis of breast cancer* |
| **10:30-11:00** | **Break** |
| **11:00-12:00** | **Session 5 - Cell Biology and Functional Proteomics**<br>**Chair: Prof. Nurhan Özlü**<br>**Jeroen Krijgsveld, German Cancer Research Center (DKFZ), Heidelberg University, Germany**<br>*Characterizing proteome regulation and cell signaling by nascent proteomics*<br>**Dominic Winter, Bonn University, Germany**<br>*Proteomic characterization of lysosomal composition, structures, and interactions* |
| **12:00-13:00** | **Satellite Symposium**<br>**Bruker / Terra Analiz**<br>**Valeriia Kuzyk - Field Application Scientist, Bruker**<br>*In-depth and high-throughput proteome analysis enabled by 4D-proteomics™ ecosystem* |
| **12:00-13:30** | **Lunch Break / Poster Session** |

| Time | |
|---|---|
| 13:30–14:30 | **Session 6 – Multiomics Approaches**<br>**Chair: Prof. Talat Yalçın**<br>**Nurcan Tunçbağ, Koç University, Türkiye**<br>*Network medicine: Leveraging integrated connections of multi-omic data*<br>**Ahmet Okay Çağlayan, Dokuz Eylül University, Türkiye**<br>*Rare diseases in the multiomic era: Malformations of cortical development as an example* |
| 14:30–14:45 | **Break** |
| 14:45–15:45 | **Session 7 – Proteomics in Model Organisms / Bioinformatics**<br>**Chair:  Prof. Gizem Dinler Doğanay**<br>**Güneş Özhan, İzmir Biomedicine and Genome Center, Türkiye**<br>*Unraveling the regulatory mechanisms underlying Wnt-receptor complex formation using the zebrafish model*<br>**Daniel J. Geiszler, Koç University, Türkiye**<br>*Illuminate your open search: Comprehensive PTM identification* |
| 15:45–16:30 | **Career Panel**<br>**Chair: Prof. Oğuz Altungöz**<br>**Nurhan Özlü, Koç University, Türkiye**<br>*Networking opportunities in Proteomics*<br>**Jale Şahin, The Scientific and Technological Research Council of Türkiye (TÜBİTAK)**<br>*EMBO and ICGEB scholarship and funding program* |
| 16:30–16:45 | **Break** |
| 16:45–17:45 | **Oral Presentations**<br>**Chair:  Assoc.Prof. Nurcan Tunçbağ**<br>**Meltem Kaya**<br>**S08** *Quantification of DNA repair proteins in glial tumours by LC/ID-HR-MS*<br>**Hasan Ufuk Celebioglu**<br>**S09** *Effects of exoproteome from lactobacillus acidophilus on colon cancer cells*<br>**Hacı Mehmet Kayılı**<br>**S10** *Diagnosis of stomach cancer using clinical glycomics and machine learning*<br>**Günnur Güler**<br>**S11** *Optical spectroscopy in proteolysis: Label-free tracking of protein alterations*<br>**Melike Dinç**<br>**S12** *Contribution of peptide fractionation to proteomic profiling by orbitrap fusion* |
| 17:45–18:15 | **Award Ceremony and Closing** |

***All sessions will be in English, simultaneous translation will not be available.**

# ORAL PRESENTATION ABSTRACTS

## S01
## Palmitoylation Directs PCDH7 to Plasma Membrane During Cell Division

Nazlı Ezgi Özkan Küçük[1], Berfu Nur Yiğit[2], Beste Senem Değirmenci[2], Altuğ Kamacıoğlu[2], Nurhan Özlü[2]

[1] Koç Üniversitesi, Translasyonel Tıp Araştırma Merkezi
[2] Koç Üniversitesi Moleküler Biyoloji ve Genetik

**Aim**: Plasma membrane changes during cells division and understanding the molecular details of those alterations is crucial to understand the division process. We previously reported that PCDH7 gets enriched at the plasma membrane in a mitosis dependent manner. In this study, our aim was to identify possible interaction partners of PCDH7 that can reveal the mechanism underneath the cell cycle dependent cell surface enrichment.

**Methods**: We took BioID, proximity dependent biotinylation approach followed by LC-MS/MS to identify the cell cycle specific proximity interactors of PCDH7. Among those interactors, a palmitoyltransferase was identified, suggesting the palmitoylation of PCDH7. We further combined mass spectrometry results with biochemical and microscopy approaches to examine palmitoylation dependent surface localization of PCDH7 during the cell cycle.

**Results**: BioID map, suggested that palmitoyltransferase ZDHHC5 was as one of the proximal interactors of PCDH7.  By taking acyl-biotin-exchange approach, we showed that PCDH7 gets palmitoylated. Pull down assay showed that PCDH7 interacts with the ZDHHC5. Both PCDH7 and ZDHHC5 co-localize at the mitotic cell surface, and they translocate to the cleavage furrow during cytokinesis. Knockdown of ZDHHC5 affects both surface and cleavage furrow localization of PCDH7. Moreover, ZDHHC5 knockdown disturbs cell division and results increased multinucleation.

**Conclusion**: Our mass spectrometry analysis revealed the interaction of PCDH7 with palmitoyltransferase ZDHHC5. As confirmed by the biochemical and microscopy assays, this interaction is required for the mitosis specific plasma membrane enrichment of PCDH7. Here we represent the very first example on how palmitoylation has a cell cycle dependent regulatory role for the localization of a protein. Given the reversibility of this relatively overlooked post translational modification, palmitoylation may have key functions during cell division. Our future work will focus on revealing the cell cycle specific palmitoylation network to dissect these functions.

## S02
## Nek2 Regulates Multipolar Spindle Formation in Centrosome Amplified Cancer Cells

Selahattin Can Özcan[1], Batuhan Mert Kalkan[2], Enes Çiçek[2], Ceyda Açılan Ayhan[3]

[1] Koç University, Research Center For Translational Medicine
[2] Koç University, Health Sciences Institute
[3] Koç University, School Of Medicine

Unlike normal cells, cancer cells frequently exhibit extra centrosomes, which tend to form multipolar spindles (MPS), triggering cell death. Nevertheless, cancer cells divide successfully by clustering their extra centrosomes into two poles. Given that Nek2 kinase is a key enzyme regulating mitotic processes including centrosome cycle, we tested whether Nek2 has a role in centrosome clustering in addition to its known role in centrosome separation.

First, we checked how Nek2 over-expression regulates MPS generation in N1E-115 cells, which harbors endogenous extra centrosomes, and observed that Nek2 over-expression decreased centrosome clustering. Then, we used two chemical (nocodazole and dihydrocytochalasin B) and two genetic (PLK4 and STIL over-expression) methods to amplify centrosomes in U2OS and MDA-MB-231 cells, and tested the role of Nek2 on MPS generation. We observed that overexpression of Nek2 resulted with unclustering of extra centrosomes and lead to MPS, while reduction of endogenous Nek2 levels re-clustered the poles, leading bipolar divisions. Loss of function experiments of previously identified Nek2 targets as TRF1, C-Nap1, GAS2L1 and Rootletin showed that formation of Nek2 induced MPS generation is independent from tested targets.

To better understand the mechanism of Nek2 induced MPS generation, we used TurboID proximity labeling and proteomics to identify novel interaction partners of Nek2 and identified several promising proteins, which have roles in different centrosome clustering mechanisms, as NUMA1, KIFC1 and KIF2C. Co-immunoprecipitation experiments further confirmed the interaction between Nek2 and KIF2C, and loss of KIF2C prevented cells from Nek2 induced MPS generation.

Taken together, we show that Nek2 regulates MPS formation in cancer cells with centrosome amplification through interacting with KIF2C.

**S03**

# Detection and Functional Analysis of CHEK2 VUSes in Cancer Patients

Nazlı Dilara Erdoğdu[1], Nisan Denizce Can[1], Celil Mert Gül[1], Nihat Buğra Ağaoğlu[2], Hamdi Levent Doğanay[3], Gizem Dinler Doğanay[1]

[1] Istanbul Technical University, Department Of Molecular Biology And Genetics
[2] Ümraniye Research And Teaching Hospital, Department Of Cancer Genetics
[3] Bahcesehir University, Department Of Gastroenterology

**Background/aim:** CHK2 (Checkpoint Kinase 2) is a signal transducer kinase and activated by the ATM/ATR pathway as a result of double strand DNA breaks. CHK2 activation triggers apoptosis, cell cycle arrest, and DNA repair processes. Due to its critical function in the DNA damage checkpoint, several inherited mutations of *CHEK2* are recognized as being harmful. This study aims to examine the impact of recurrent *CHEK2* variations of unknown significance (VUS) on CHK2 structure and function in individuals with breast and colorectal cancer.

**Methods:** 1294 breast cancer patients, 370 colorectal cancer patients, and 490 healthy individuals from Turkish population were screened for cancer susceptibility genes. Mutations on *CHEK2* gene were classified according to ACMG guidelines to detect recurrent VUSes. Wild-type and four selected CHK2 VUSes were produced in *E. coli* BL21 cells and purified using immobilized metal affinity chromatography (IMAC). Secondary structures of purified proteins were examined using circular dichroism. In addition, to observe the stability and function of CHK2 and one of its downstream targets p53, wild-type and mutants were overexpressed in MCF-7 breast cancer cell line.

**Results:** Wild-type and mutant CHK proteins were successfully produced in *E. coli* BL21 cells, and purified. Secondary structures of purified proteins revealed that all mutants except L183F conserved their native structure as mainly revealing alpha helices. In MCF-7 cells, an intriguing alteration on both CHK2 and p53 stability was observed for L183F overexpressed cells.

**Conclusion:** As a result of genetic screening of 2154 individuals, out of the most recurrent four *CHEK2* VUSes, L183F mutation resulted in altered protein structure and function. The further *in vitro* activity assays, protein-protein interaction and stability analyses will be useful to discover the potential effects of mutations on protein function.

## S04
## Analysis of the İnteraction Between Thymoquinone and Oncogenic Driver Proteins

Aycan Sezan[1], Senanur Taş[1], Ege Özkan[2], Mojahidur Hasan[3], Sehreen Tory[3], Yağız Çapanoğlu[1], Burcu Saygıdeğer Demir[1], Yasemin Saygıdeğer[4]

[1] Department Of Biotechnology, Institute Of Natural And Applied Sciences, Cukurova University
[2] Department Of Bioinformatics And Genetics, Faculty Of Engineering, Kadir Has University
[3] Department Of Translational Medicine, Institute Of Health Sciences, Cukurova University
[4] Department Of Pulmonary, School Of Medicine, Cukurova University

**Aim:** Thymoquinone (TQ/2-methyl-5-isopropyl-1,4-benzoquinone) is a phytochemical produced from black seed (Nigella sativa) used in traditional medicine in the Middle East, Mediterranean, South Asia, and Africa. In vitro and in vivo preclinical research showed TQ's antidiabetic, analgesic, antihypertensive, antimicrobial, anti-inflammatory, bronchodilatory, gastroprotective, hepatoprotective, immunomodulatory, spasmolytic, renal-protective, antioxidant, and anticancer properties. Recent studies demonstrate it suppresses cancer cell growth in breast, colon, larynx, lung, myeloblastic leukemia, osteosarcoma, ovary, and pancreas cell lines. In this study, we analyzed the structural interaction between oncogenic driver proteins and TQ-targeted treatment.

**Methods:** The 3D structure of the ligand (TQ) was obtained from PubChem and converted to PDB format using the software Discovery Studio 2016 (BIOVIA). The PDB files of the proteins to be analyzed were obtained from the Protein Data Bank, and Autodock Vina was used to marking the active site for docking analyses. Using Discovery Studio programs, images of protein-ligand interactions were also created. The results were compared with the online analysis program Swissdock.

**Results:** The studied proteins were EGFR, KRAS, P53, TP63, ROCK1, CD44, ALDH1A, HIF1alpha, HER2, PTEN, GLUT1, JAK1, and TGFbetaR2. KRAS G12C had the lowest Binding energy values with TQ (Table-1), but this interaction had an unfavorable acceptor-acceptor interaction with TYR _32)that could affect the stability of the ligand-protein interaction. Multiple Pi-Sigma interactions occurred between TQ and JAK1, EGFR T790M, P53, and HIFA (Figure-1). HER2 formed the only covalent bond whose binding energy was compatible with this interaction (Figure-2).

**Conclusion:** The results of this in-silico study showed that TQ might be interacting with several oncogenic proteins to possess its anticancer activity and HER2 was found as the most potent target of this compound.

## S06
## Regulation of Keratin 8 Phosphoprotome During Cancer Cell Division

Ceyda Seren Ceyhan, Ali Yurtseven, Aybaran Olca Kebabcı, Büşra Aytül Akarlar, Nurhan Özlü

Koç Üniversitesi, Moleküler Biyoloji ve Genetik Bölümü

**Background/aim:** Keratins are intermediate filaments distributed in epithelial cells. They have diverse expression patterns in different cells and are used as markers in diagnostic tumor pathology. However, it is not well studied how these robust structures do not prevent cell division and how they are regulated during dynamic processes, such as cytokinesis. It is known that phosphorylation on Keratin proteins increases their solubility and alters their phenotype. In our study, we focus on Keratin 8 and aim to investigate its plasticity during cancer cell division by searching its phosphorylation profile.

**Methods:** MCF7, HeLa.S3, MDA-MB-231, and CAKI-2 cancer cells are arrested at interphase, mitosis, and cytokinesis stages. Phosphorylation profiles are investigated by label-free and stable isotope dimethyl labeling-based quantitative phosphoproteomics approaches. To reduce the complexity of the peptides, samples are fractionated by Strong Cation Exchange Chromatography and low abundant phospho-peptides are enriched with $TiO_2$ beads. Enriched phospho-peptides are subjected to LC-MS/MS analysis, and raw data files are processed with PD 2.3 and MaxQuant software.

**Results:** Cell cycle stage-specific phospho-sites of K8 are analyzed for each cell line. By comparing label-free and quantitative analysis methods, the specificity of the phosphorylation sites to cell cycle stages is examined. Inter-cell type comparisons revealed commonly regulated phospho-sites in all cell lines at the head domain of Keratin 8. According to intra-cellular phosphorylation analysis, MCF7 cells have the highest number of phospho-sites with the highest Keratin 8 expression levels. The number of phospho-sites decreases as the Keratin 8 expression levels decrease through epithelial to epithelial-mesenchymal cells.

**Conclusion:** Our findings revealed upregulated phospho-sites of Keratin 8 at specific cell cycle stages. There are commonly regulated phospho-sites in all cell lines but also cell type unique ones that are potential marker candidates for cell division and tumor diagnosis.

## S07
## Equine Proteomics: A Scoping Study of Current Trends and Future Aspects

Sinan Kandır[1], Fatıma Uzan[2], Tuğrulhan Özden[2]

[1] Cukurova University Ceyhan Faculty Of Veterinary Medicine Department Of Physiology
[2] Cukurova University Institute Of Natural And Applied Sciences Department Of Biotechnology

**Purpose:** Early diagnosis, intervention, prevention, and treatment in veterinary medicine are crucial and strongly correlated with public health. In equine medicine, proteomics is used for basic research and diagnostic tools for various diseases. Thus, we aimed to scope the studies with current literature knowledge and prospects the future studies and clinical applications.

**Methods:** The study was methodologically designed with scoping studies frameworks[1,2]. The search terms were "equine proteome," "equine proteomic," "equine proteomics," "horse proteome," "horse proteomic," and "horse proteomics" in the MEDLINE/PubMed, Cochrane Database, CAB Abstract, and Google Scholar with "AND" and "OR" the defining terms was added as logical operators as "mare" and "stallion." No time and language restrictions were applied, though the reviews were excluded. A total of 312 articles were evaluated by the authors independently, and after discussion, a common consensus was achieved. The 196 research articles were included in the scoping study. The articles were evaluated and analyzed by web-based Rayyan by scoping review type[3].

**Results:** The first publications emerged in the early 2000s, which encountered the post-genomic era and are still rising to the present. Even though the research topics are diversified from in vitro to food security, most equine proteomic research concentrated on reproductive (%30) and musculoskeletal systems (%12). Body fluids are distinguished sources, and 2D/3D gel electrophoresis, LC-MS/MS, and MALDI-TOF techniques are used to evaluate the proteomic profiles.

**Conclusion:** New disease-specific biomarkers, pharmaceuticals, and treatment strategies are needed to formulate early diagnosis procedures and differentiate diseases with similar clinical characteristics. However, proteomic analyses are still not practical and fully adopted in veterinary clinics due to their sensitive and intensive labour with expertise. Nonetheless, the omics era has surrounded a respectable audience of clinicians.

## S08
## Quantification of DNA Repair Proteins in Glial Tumours by LC/ID-HR-MS

Gamze Tuna[1], Meltem Kaya[1], Hande Oğuzhan[1], Sertaç İşlekel[2], Hüray İşlekel[1]

[1]Dokuz Eylül Üniversitesi Sağlık Bilimleri Enstitüsü Moleküler Tıp Ana Bilim Dalı
[2]İzmir Medicana Hastanesi

**AIM**: Glial tumors are the most frequent primer malignant tumors on central nervous system and derived from glial cells. DNA of the living cells is constantly damaged by endogenously or exogenously and most of the DNA lesions are repaired by base excision repair (BER) pathway. Human apurinic/apyrimidinic-endonuclease-1 (hAPE1) is a multifunctional protein that plays a central role in the cellular response to free radicals and oxidative stress. Human poly (ADPribose)-polymerase-1 (hPARP1) plays a major role in the repair of DNA strand breaks. It is known that DNA repair capacity increases in malignant tumours and it has a positive correlation with drug resistance. For these reasons, the main purpose of this study is to identify and quantify hAPE1 and hPARP1 protein in glioma patient tissue sample with liquid chromatography/ isotope-dilution high resolution mass spectrometry (LC/ ID-HR-MS) method.

**Method**: Proteins were extracted from tissue sample and digested with trypsin. hAPE1 and hPARP1 were analyzed by LC/ ID-HR-MS. A fully 15 N-labeled analogue of hAPE1 and Lys-13C6, 15N2– or Arg13C6, 15N4– labeled tryptic peptides of hPARP1 were used for the quantitative measurements. Peptides were defined and mass calculations were made as much as the number of peptides calculated at the significance level $p < 0.05$.

**Results**: Six peptides for hAPE1 and five peptides for hPARP1 were identified which matched to a subset of the theoretically predicted tryptic peptides of hAPE1 and hPARP1. The amount of hAPE1 and hPARP1 proteins were calculated at the level of ng/µg protein in the glial tissue sample.

**Conclusion**: With this study, a targeted bottom-up proteomics approach was developed for hAPE1 and hPARP1 measurements in human glial tissue sample.

## S09
## Effects of Exoproteome From Lactobacillus Acidophilus on Colon Cancer Cells

Hasan Ufuk Celebioglu[1], Yavuz Erden[2], Busenur Celebi[1], Ebru Koroglu[1], Rizvan Imamoglu[2]

[1] Bartin University, Faculty Of Science, Department Of Biotechnology
[2] Bartin University, Faculty Of Science, Department Of Molecular Biology And Genetics

**Aim:** Probiotics are microorganisms having positive effects on human health when taken in adequate quantities. *Lactobacillus acidophilus* is among the most studied probiotic bacteria and effective in preventing cancer cells but exact molecular mechanisms have not yet been fully elucidated.
In previous studies, the proteins secreted by such microorganisms outside the cell (Exoproteome) play important roles in probiotic effects like adhesion to the mucus layer and strengthening the immune system, but their effects on cancer cells have not been determined at molecular level. Thus, the present study aimed to investigate the effects of *Lactobacillus acidophilus* Exoproteome and pyruvate kinase present in Exoproteome on colon cancer.

**Method:** Exoproteome and Recombinantly produced Pyruvate Kinase of *Lactobacillus acidophilus* were applied to human colon cancer cell lines HT-29 and Caco-2. MTT method and "Comet Analysis" were used for cytotoxicity and genotoxicity, respectively. Furthermore, morphological changes in cells were revealed using a scanning electron microscope (SEM).

**Results:** The results showed that Exoproteome has a cytotoxic effect on both HT-29 and Caco-2 cells. The cytotoxic effect is thought to occur by inducing apoptosis according to SEM analysis because the morphological changes in cells resemble the morphology of typical apoptotic cells. Furthermore, while pyruvate kinase reduced the viability of HT-29 cells by around 10% ($p<0.05$), this protein was found not to affect Caco-2 cells. Genotoxicity and SEM analyzes also support these findings.

**Conclusion:** As a result, it is thought that the effect of *Lactobacillus acidophilus* on cancer cells may be mediated by its Exoproteome, but pyruvate kinase, which is also in this Exoproteome, is generally not one of the factors responsible for this effect even though it affected HT-29 cells to a relatively lesser extent.

## S10
## Diagnosis of Stomach Cancer Using Clinical Glycomics and Machine Learning

Deniz Baran Demirhan[1], Hakan Yılmaz[1], Harun Erol[2], Hacı Mehmet Kayılı[1], Bekir Salih[3]

[1]Karabuk University, Faculty Of Engineering, Biomedical Engineering Department
[2]Karabuk University, Faculty Of Medicine, Pathology Department
[3]Hacettepe University, Faculty Of Science, Chemistry Department

**Aim:** Stomach cancer is one of the most common types of cancer in the world. The number of studies in the literature on the examination of N-glycosylation structures on gastric cancer tissues is very few. This study aims to develop a new application for diagnosing stomach cancer from FFPE tissue sections using clinical glycomics and machine learning algorithms.

**Method:** FFPE gastric cancer and adjacent control tissues were taken from the Pathology Department of Karabük Training and Research Hospital. Extraction of proteins from tissues was carried out with the chloroform/methanol extraction method. After the release of N-glycans from the extracted glycoproteins, the glycans were labeled with a 2-AA tag. N-glycans were analyzed by MALDI-TOF-MS after their purifications. The relative areas of the glycans detected as a result of these analyzes were calculated. Data obtained from a maximum of 4 technical replicates of each cancer and control sample were included in the analyses.

**Results:** As a result of the analysis, 59 N-glycans were detected and structurally confirmed by MALDI-MS. Significant results were obtained from the data by using KNN, RF, DT, SVM, LR, and MLPC machine learning algorithms. It was observed that there were significant differences between cancer and control tissues in N-glycans with applied algorithms and model evaluations. The MLPC algorithm provided the highest score in each data set. In the neutral N-glycans relative area dataset, the accuracy score of the MLPC algorithm was 93.6%+-0.8%, and the ROC analysis was found to be 0.99.

**Conclusion:** A new application for the diagnosis of stomach cancer from FFPE tissue sections was developed using glycomics data sets with machine learning algorithms.

## S11
## Optical Spectroscopy in Proteolysis: Label-Free Tracking of Protein Alterations

Günnur Güler

Izmir Institute Of Technology

**Aim:** Proteolysis, the enzymatic hydrolysis of peptide bonds in proteins/polypeptides, is of wide interest for many fields including biology, biotechnology and biosciences. In the current study, our aim was to develop a quantitative model of proteolysis and to elucidate its functional mechanism by following the protein secondary structural alterations and micellization.

**Materials and Methods:** Proteolysis process was evaluated by the example of proteolysis in aqueous-organic media ($H_2O$/alcohol) and in deuterium oxide ($D_2O$). Ambient temperature and the ratio of substrate-to-enzyme (S:E) was lowered. The enzymatic reaction of various substrates (native/gently modified globular/non-globular proteins) with trypsin was followed by using optical spectroscopic techniques such as mid-infrared (IR) spectroscopy (Perkin Elmer UATR Two, UK) and far-UV Circular Dichroism (CD) spectroscopy (J-1500, Jasco).

**Results:** Based on the vibrational mid-IR data, the most considerable spectral alterations caused by enzymatic attack are displayed in the amide I and amide II regions (1700-1500 $cm^{-1}$), which arise mainly from protein secondary structures ($\alpha$-helix, $\beta$-sheet, turn/loops, random coils). Since mid-IR spectroscopy is sensitive to molecular vibrations of functional groups, the IR signals of liberated both $NH_2$ and carboxylate groups are detected at 1613 $cm^{-1}$ and 1595/1420 $cm^{-1}$, respectively, as proteolysis products. Two-dimensional correlation spectroscopy (2DCOS) analysis reveals the sequential order of events in the intact proteins during enzymatic attack. Both vibrational mid-IR and electronic CD data provided label-free following of secondary structural alterations (rearrangement/degradation) of the protein substrates during proteolysis.

**Conclusion:** Development of a quantitative model of proteolysis becomes increasingly important for the biotechnological applications and proteomics. Functional mechanism of proteolysis will be investigated further by using the biophysical/physicochemical methodologies. This work was funded by the Scientific and Technological Research Council of Turkey through the international TUBİTAK-2532 Russia Bilateral Cooperation project (Grant#119N423 to Dr. G. GÜLER).

## S12
## Contribution of Peptide Fractionation to Proteomic Profiling by Orbitrap Fusion

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**Purpose:** To evaluate peptide fractionation method compared to single-shot LC-MS/MS analysis

**Methods:** Shotgun proteomic analysis was applied to a sample of Leishmania parasite by using off-line high-pH reversed-phase fractionation and concatenation strategy. A total of 16 fractions of the peptide mixture was analysed individually and combined for an in-depth proteomic analysis. A mixture of equal volumes of the fractions were also run for a single-shot proteomic analysis. All data were collected using DDA acquisition by an analysis lasting 150 minutes.

**Results:** More than 2000 proteins were identified in the single-shot analysis while this number reached up to 4800 in the fractionation method. Concomitantly, protein sequence coverages were increased in many proteins.

**Conclusion:** As expected fractionation provided a lot more protein than single-shot analysis. The later was also satisfying especially for a differential proteomic analysis which requires several biological replicate. If protein number or sequence coverage were not compromised, fractionation method should be render more cost-effective by decreasing fraction number to reasonable level or by optimizing the analysis parameters.

# POSTER PRESENTATION
# ABSTRACTS

## P01
## Determination Of Bcl-2/Beclin 1 Interaction Surface Through Mass Spectrometry

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**Aim:** Anti-apoptotic B-cell lymphoma 2 (Bcl-2) is a member of Bcl-2 family, which is composed of structurally related proteins controlling intrinsic apoptosis, and responsible for control of intrinsic apoptosis initiation in mitochondria. Beclin 1, a Bcl-2 interacting protein, is the main component of PI3K complex and is accepted as the central regulator of macro-autophagy. Interaction of Bcl-2 with BH3 domain of Beclin 1 prevents Beclin 1 from forming pre-autophagosomal complex and inhibits autophagy initiation. Hence, Bcl-2/Beclin 1 complex generates a decision point between apoptosis and autophagy pathways. Although main mechanisms controlling their interaction were widely studied, structure of their interaction surface still remains unknown.
In this study, our aim is to probe putative binding interface of Bcl-2 and Beclin 1. Determination of this surface may provide another druggable sites which can be targeted by anti-cancer drugs.

**Methods:** Full-length Bcl-2 and Beclin 1 proteins were produced in HEK293T mammalian cells via transient transfection and affinity purified through Flag-tag or His-tag, respectively. Activity of proteins was verified *in vitro* via both on-bead interaction assay and membrane immobilized *in vitro* interaction. Upon confirmation, interaction region of Beclin 1 with Bcl-2 in Bcl-2/Beclin 1 complex was revealed by membrane immobilized *in vitro* interaction assay followed by limited tryptic digestion. Acquired peptides were analyzed and identified by LC/MS/MS.

**Results:** Bcl-2 and Beclin 1 proteins were successfully produced in mammalian cells and purified with >80% purity. Interaction of recombinantly Bcl-2 and Beclin 1 was verified with *in vitro* interaction methods. Peptides from interaction region of Beclin 1 with Bcl-2 in Bcl-2/Beclin 1 complex was identified with LC/MS/MS. ECD and BH3 domains were found to be the key interaction points.

**Conclusion:** According to our data, ECD domain of Beclin 1, in addition to the BH3 domain, is critical for interaction with Bcl-2.

## P02
## Monitoring Of Aberrant Proteins In Breast Cancer Cells Through Retrotranslocation

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**Background/aim:** Protein quality control (PQC) system is activated directly, when newly synthesized proteins emerge from ER lumen and chaperones assist proteins for their folding and prevent any misfolded proteins from escaping PQC. Fully folded proteins can leave the ER lumen along the secretory pathway, while misfolded/unfolded proteins are transferred back into cytosol by process cascade called retro-translocation and ER-associated protein degradation (ERAD). Derlin1 is the major component of retrotranslocon that is ER-membrane embedded cavity. Degradation of both soluble and integral membrane ERAD substrates requires Derlin1. In our study, we aimed to monitor cell-specific aberrant substrates of Derlin1 in breast cancer cell lines via LC-MS/MS.

**Materials and Methods:** Derlin1 fused biotin ligase was cloned into mammalian expression system. Cell culture assays were conducted in breast cancer cell lines such as MCF-7 and MDA-232. Overexpression and biotinylation levels were examined with immunoblotting. *In-situ* localization of biotin ligase fused Derlin1 was confirmed via immunocytochemistry (ICC). Biotinylated proteins were captured with streptavidin pull-down and treated by trypsin enzyme for further identification via LC-MS/MS (Waters Synapt G2-Si). Analysis of LC-MS/MS data were performed with ProteinLynx Global Server software (PLGS, Waters).

**Results:** Biotin ligase fused Derlin1 was detected as colocalized with ER-membrane resident protein Calnexin via confocal microscopy. Immunoblotting results showed Derlin1 fusion protein was overexpressed and biotinylated its interaction partners. Cell-specific ERAD associated novel interaction partners were found in LC-MS/MS based protein identification.

**Conclusion:** According to preliminary data, identification novel aberrant Derlin1 substrates are promising drug candidates for specific breast cancers.

## P03
## Diverse Molecular Functions Regulated By Nek2 Kinase: A Proteomic Study

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**Aim**: Targeted therapies demonstrate greater promise in cancer treatment, in comparison to conventional methods such as chemotherapy. Nek2 kinase is one of the attractive targets in cancer therapeutics due to its key functions in cell cycle, proliferation, and drug resistance. Comprehensive understanding of complex biological systems enables researchers and clinicians to achieve accurate predictions on functional outcomes of targeting key molecules. Exploiting omics-based technology and patient databases, biological changes driven by overexpression or suppression of a specific protein and their clinical relevance can be revealed.

**Method**: We utilized global proteomics and bioinformatics tools to identify the changes in proteome and molecular pathways depending on the expression levels of Nek2 in cancer cells. We used doxycylin inducible Nek2 overexpression system and siRNA to manipulate expression levels of Nek2 in U2OS cells.

**Result**: Amongst 1815 total detected proteins, we determined 358 differentially regulated proteins with statistical significance and minimum 20% change in abundance. Combining our LC-MS/MS data and cancer patient datasets, we report that KIF20B and RRM1 levels are highly correlated with Nek2 expression. Nek2 silencing results in significant reduction in both KIF20B and RRM1 protein abundance, in addition, we identified potential phosphorylation sites by Nek2 kinase.

**Conclusion**: Collectively, our data suggests that KIF20B and RRM1 could be either potential solo targets for therapeutic approaches or in combination with Nek2 inhibitors to improve clinical success.

## P04
## TurboID Labelling Reveals Cell Cycle Dependent Interactome Of Nek2A Kinase

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Nek2A is a cell cycle regulated kinase, which is involved in several cellular processes and overexpressed in numerous cancer types. It has been associated with several drivers of tumorigenesis including chromosome instability, increased cell proliferation or drug resistance. In order to explain these outcomes, our laboratory has previously shown that overexpression of Nek2A can lead to multipolar mitosis via unclustering of extra centrosomes. The best characterized role of Nek2A is its phosphorylation of centriolar linkage proteins in the G2/M phase of the cell cycle.

With the aim of defining new partners of Nek2A, we used Nek2A-TurboID proximity labelling system in synchronized cell populations and determined the changes in the Nek2A interactome in G1/S, late S, or G2/M phases of the cell cycle. The expression and localization of TurboID-Nek2A-Wild Type and -Kinase Dead proteins were confirmed by both western blotting and immunofluorescence staining. For cell syncronization, double thymidine was used, and cells were collected at time points corresponding to different cell cycle stages. The efficiency of synchronization was determined by flow cytometry using PI staining. Following biotinylation, samples were sent to spectrometric analyses via Orbitrap mass analyzer, and the results were evaluated by MaxQuant and Cassopeia softwares. Confirming the robustness of our system, several known Nek2A targets, such as LRRC45 and KIF24, and proteins known to act on Nek2A such as ANAPC1 or ANAPC4 were identified. In addition to these known interactors, many other novel proteins were found to be significantly enriched by 3 fold or more. Among these, we focused on KIF2C, NUSAP1, MAPRE3 and MPG, based on their known function related to cell cycle or spindle formation. Currently, we are investigating these proteins both for the nature of their interaction with Nek2A and also for their potential involvement in Nek2A mediated multipolar divisions during cell cycle.

## P05
## Divalent Ions Affect The Interactions Between Hıstones and DNA

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In eukaryotes, ˜147 bp of DNA is packaged around histones (H2A, H2B, H3, H4) corresponding to helices (a1, a2, and a3). These helices interact to form the octamer called nucleosome, the basic repeating unit of chromatin. The anchoring of basic lysine and arginine side chains inside the histones into the minor grooves of DNA facilitates the interaction between DNA and histones. The epigenetic substitutions inside the histones, such as bulky arginine to compact lysine substitution in H3 (CENP-A formation), might fine-tune the affinities of histones to DNA. The interactions for the folding of nucleosomes might depend on magnesium concentration inside the cell.

To analyze the effect of this concentration on the interaction between DNA and lysine and arginines inside the helices, we prepared two Widom601 sequence regions interacting with a CENP-A region and a canonical H3 region, respectively, via Pymol. Then, the energies of these molecules were minimized and the molecules were resolved in the cubic OPC water box for 1 ms, separately, via CHARMM36 force field and Gromacs, while $Na^+$, Cl, and $Mg^{++}$ ions were providing a physiological condition. The simulations were replicated in a condition with excess $Mg^{++}$. The trajectories were obtained and analyzed via VMD and Python.

It was revealed by contact analysis that magnesium induces the detachment of histones, especially CENP-A, from DNA. Moreover, the distance of CENP-A to DNA is increasing significantly in a high divalent environment, compared to canonical H3. Lastly, root mean square fluctuation analysis of histones shows that the stability of canonical H3 is less than CENP-A in both physiological and high divalent conditions, and increased magnesium concentration decreases the stability of these histones.

This study reveals that an excess amount of divalent ions affects the stability of histones with regards to sequence, and the binding interactions of these histones and DNA.

## P06
## In-Silico,Characterization Of A Rare-Disease That Affects The Dynamics,Function Of Linker-Histone-H1

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**Aim**: The eukaryotic genome is wrapped in nucleosomes to form chromatin. Nucleosomes contain 147 bp of DNA wrapped ~1.65 times around a histone octamer comprising two copies each of the core histones H2A, H2B, H3, and H4. In higher eukaryotes, a fifth histone, known as the linker histone H1, binds the nucleosome and the linker DNA between nucleosomes to facilitate the folding of the chromatin fiber into a higher-order three-dimensional structure.
In late 2019, germline frameshift mutations involving one of the H1 subtypes, H1.4, were causally linked to an as-yet poorly defined syndrome called the Rahman syndrome (RS). In affected individuals, characteristic phenotypes include intellectual disability and skeletal and cardiac anomalies. We hypothesize that the chromatin domains harboring the RS-H1.4 could exhibit a less condensed and differently organized structure, which could disrupt the delicate architecture and function of chromatin.

**Material and Methods**: To understand how the RS mutant H1.4 affects nucleosome dynamics, we performed atomistic molecular simulations of the WT (wild-type) and the RS-H1.4 bound nucleosome. Among all RS patients, we have simulated the most affected RS-H1.4 from "patient#1".

**Results**: Indeed, we found that the H1.4 of patient#1 could not only not fold the linker DNA strands but was destabilizing them in comparison to the WT as well as a "tailless" mutant that lacks the CTD.

**Conclusion**: In conclusion, the findings of this study will not only give us information about the RS but also provide critical findings about the 3D chromatin architecture mechanism. The combination of these studies is expected not only to shed light on the mechanism of RS but could also be used as an example of a "workflow" for rare disease studies.

## P07
## Characterization Of Human Linker-Histone Isoforms In Nucleosome-Bound State At Atomistic-Level

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**Aim**: The nucleosome is the repeating unit of chromatin and it is composed of an octamer histone core that holds together around 145 base pairs of DNA. In higher eukaryotes, an additional histone, linker histone H1, provides further structural order by stabilizing the nucleosomal DNA. H1 family includes seven somatic variants and four variants expressed only in germ cells, three are testis-specific, and one is oocyte-specific. These isoforms are expressed differentially across cell types and stages, suggesting diverse functional roles in various physiological processes including gene expression, differentiation, aging, and cancer.

**Material and methods**: We employed molecular modeling and molecular-dynamics simulations to gain insights into the dynamic behavior of these sex-specific isoforms in free- and chromatin-bound states. For isoforms where an experimental structure is not available or is not sufficiently reliable, we used AlphaFold predicted structures and SuperLooper2 structural corrections. We collected production trajectories using Gromacs molecular dynamics suite used VMD and Python scripts for analysis.

**Results**: During molecular dynamics simulation of H1.8 isoform in chromatin-bound state, we observed loss of secondary-structure features. To resolve this, we generated different starting models of H1.8 using conventional modelling approaches as well as the more recent machine-learning based prediction tools. Among all the structural models, AlphaFold predicted initial structure provided the best overall stability, excelling not only in comparison to original, cryo-EM experimental model but also in comparison to the conventional molecular modelling proposals.

**Conclusion**: Encouraged by this result, we are now exploring the possible binding modes of the testis-specific isoform to chromatin, for which only machine-learning predicted atomic models are available ,in isolated state. This endeavor is beneficial not only from a computational structural biological standpoint but also in terms of validation of machine-learning based protein structure predictions which yet lack a consideration of the effect of binding partners.

## P08
## Production Optimization And Characterization Of Anti-RBD Nanobody TY1

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**Background/Aim:** The COVID-19 pandemic has been affecting the world since 2019. Although the progress of the pandemic was controlled with vaccines, the new variants that have emerged make it necessary to develop agents that will prevent the virus from infecting the host cell. One of the potential treatments against SARS-CoV-2 is inhibition of the binding of receptor binding domain (RBD) of the Spike S surface protein on virus with the Angiotensin 2 (ACE2) receptor of the host cell. Antibodies targeting the disruption of this interaction have been developed using antibodies, however, the large structures of the antibodies and numerous post-translational modifications, and the production requirement to mammalian cell culture, which is more costly than bacterial culture, make them disadvantageous. Nanobodies, an innovative alternative to antibodies, are the antigen binding site of heavy chain antibodies found in camelids are devoid of these disadvantages. Also, they are easily modifiable against new variants of the virus. TY1 nanobody is known to inhibit the RBD-ACE2 interaction, from that motivation we wanted to first produce TY1 and later develop its alternate nanobodies to inhibit RBD-ACE2 interaction.

**Method:** Bacterial production optimization of TY1 nanobody was carried out by screening parameters such as production temperature, IPTG concentration, and induction time. Analysis of results was performed using SDS-PAGE and immunoblotting. After production optimization, the folded structure of the purified molecule was analyzed with circular dichroism (CD) and its binding to RBD was investigated by a pull-down binding assay.

**Results:** The optimum production conditions were determined. It was observed that the purified TY1 nanobody was in a folded structure with CD and was found to be in an active form that could bind with RBD.

**Conclusion:** In conclusion, the production of anti-RBD TY1 nanobody has been optimized in *E. coli* and is to be active and folded.

## P09
## Production Optimization Of Nanobody H11-H4 To Block SARS-CoV-2 Infection

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**Background/Aim:** The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to affect public health globally. Pharmaceutics such as vaccines developed to block infection are usually antibody-based and are found to inhibit interaction of the RBD domain of Spike protein on the virus surface with the Angiotensin 2 (ACE2) receptor on the host cell surface. However, since antibodies are large molecules and their production is costly, they have been replaced by small molecules called nanobodies. Because they are smaller and more stable than antibodies, nanobodies are used in COVID-19 studies to inhibit the RBD-ACE2 interaction. In this study, we aim to optimize the conditions of the production of H11-H4 nanobody with a motivation of later improving this nanobody for the variants of the virus.

**Method:** pOPINO_NbH11-H4 was transformed into *E. coli* DH5$\alpha$ and production optimizations were performed using this bacterial strain. Then, gene encoding the 6x His-tag H11-H4 protein was taken from the pOPINO_NbH11-H4 and cloned into a bacterial pHEN vector. Constructed plasmid was transformed into *E. coli* BL21(DE3) bacterial strain for the H11-H4 protein production. Optimization studies to produce the molecule in *E. coli* was done by changing incubation temperature and inducing-agent concentration, and also, solubility of the molecule during expression was improved by osmotic shock treatment. After, H11-H4 protein was purified via ÄKTA start FPLC systems (Cytiva) using Ni-NTA column. SDS-PAGE and Immunoblotting analyses were performed to check how optimizations affect the production levels and the purity.

**Results:** SDS-PAGE and immunoblotting results have shown that anti-RBD H11-H4 nanobody was produced in *E. coli* cells at 37°C and expressed in the soluble form using osmotic shock.

## P10
## Investigation Of Bag-1S:P97 Interaction For Understanding ERAD

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**Aim:** Protein quality control (PQC) is essential to maintain proteostasis and promote cell survival. Misfolded/unfolded secretory/membrane proteins are transported from the ER to the cytoplasm via p97/VCP protein and are targeted to the UPS for degradation via ER-associated degradation (ERAD). p97/VCP is an AAA+ ATPase that allows the separation of substrate proteins from cellular structures and/or multiple protein complexes. Another component of PQC is multifunctional Bag-1S protein, which functions in the regulation of different cellular events (cell proliferation, signal transduction, stress response, protein folding, protein degradation and apoptosis) by interacting with diverse proteins. p97/VCP and Bag-1S proteins are potential targets in the treatment of various cancer types individually. Our previous studies showed that Bag-1S interacts with p97/VCP. In our broad structural biology-based breast cancer research, our aim is to investigate the Bag-1S:p97 complex to reveal their binding mechanisms and design new molecules targeting this complex through determining the druggable sites for therapeutic intervention.

**Methods:** Mammalian and bacterial expression vectors of p97 and Bag-1S proteins were cloned with N-terminal TEV-cleavable poly-Histidine tags. Mammalian and bacterial production optimizations were carried out. Afterwards, target proteins produced in bacteria were purified in sequential steps via ÄKTA FPLC systems and mammalian productions were purified via batch purification. Purity, secondary structure and oligomerization states of purified proteins were analyzed by SDS-PAGE, Circular Dichroism (CD) and SEC-HPLC, respectively. Finally, Bag-1S:p97 interaction was investigated by *in vitro* membrane spot assay.

**Results:** p97 and Bag-1S proteins were successfully purified and structurally characterized. Their interaction was confirmed with *in vitro* spot assay. Combination of HDX-MS analysis of proteins and docking analysis revaled probable interaction sites for intervention.

**Conclusion:** In the light of our ongoing studies, the interaction surface of Bag-1S:p97 complex will be determined. In this respect, it will contribute to the literature and the discovery of new target molecules.

## P11
## Thin Film Microextraction System For Biomarker Detection

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**Aim:** Lung cancer is one of the types of cancer that causes the highest number of deaths worldwide, and the diagnosis can only be made at later stages. Expensive imaging techniques applied for early diagnosis in patients at risk of lung cancer cannot provide reliable results, and these findings need to be confirmed by biopsy examinations, which are uncomfortable for the patient. Especially in the early diagnosis of lung cancer, there is an increasing number of studies showing that there are significant changes in the levels of metabolites in the urine as a result of contact with blood and that some of these substances can be used as biomarkers.

**Method:** In this study, the production of non-invasive, inexpensive, fast and specific thin-film micro-extraction (TFME) blades for the early diagnosis of lung cancer and the validation of the analysis method of metabolites from compounds that can be biomarkers with these developed blades were performed. The patient's urine samples were collected and adsorption of metabolite species with different chemical structures was performed on thin film-coated blades of different polarities integrated with the 96 well plate (96WP) system, Analyses were performed by LC-MS/MS system.

**Results:** The optimized method with coated blades was applied for metabolite determination in urine of 45 cancers and 48 asthmatic cases and the data were evaluated with chemometric techniques of diseased and healthy groups. The results of metabolite biomarkers separated from control groups in clinical samples were given.

**Conclusion:** As a conclusion, with this extraction and analysis method developed in the laboratory environment, successful treatment startegies can be applied by metabolite screening without the need for expensive treatment methods applied in diagnosis.

## P12
## Comparison Of Liquid-Liquid Extraction Strategies For Metabolite Analysis

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**Aim:** For the early diagnosis of cancer, the biological matrix of blood, sweat, saliva and urine protein groups, tumor antigens, peptides, DNA and RNA and some genes expression, and metabolic products [1] is monitored as a biomarker. The metabolomics studies of human body for biomarker selection is one of the popular studies in recent years. While the metabolomics studies in biological matrix are examined, biomarker groups are mostly analyzed by liquid and gas chromatographic techniques. The sensitivity of these techniques were improved using mass spectroscopic detectors [2-4]. However, the most important problem for the clinical use of these assays is the use of preconcentration and pre-seperation methods that can lead to desired sensitivity, support the diagnosis in the biological environment and provide the reproducibility. The main objective of this study is to develop reliable and accurate extraction method for determination of thirteen metabolites in urine samples.

**Method:** Different liquid-liquid extraction procedures using polar solvents namely, methanol, acetonitrile, dicloromethane and ethyl acetate were tested for the determination of polar metabolites by liquid chromatography tandem mass spectrometry. Multiple reaction monitoring in the negative ionization mode with ESI source was used for detection of components.

**Results:** Calibration curves were linear with correlation coefficients above 0.98. The RSD values were changed in the range of 3 – 15%. The accuracy of the method was tested with spiked urine samples and the obtained data exhibited recoveries were higher than 70% for the extraction of metabolites in urine samples.

**Conclusion:** This developed method enables methanol extraction in the urine samples of healthy and cancer patients to extract a wide variety of compounds with acceptable recoveries.

## P13
## Alpha-Tubulin Acetylation Loss Effects Golgi Morphology In SMA

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**Aim:** Spinal muscular atrophy (SMA) is a devastating and rare neurodegenerative childhood disease. It is caused by *the Survival of motor neuron 1* (*SMN1*) gene deletions, which lead to the absence of functional SMN protein. SMN protein deficiency results in impairments of the neuronal cytoskeleton, including microtubules. We previously demonstrated reduced alpha ($\alpha$) tubulin detyrosination and microtubule stability in an in vitro SMA model. Post-translational modifications of $\alpha$-tubulin, especially acetylation play a role in regulating microtubule stability. In this study, we aimed to analyze $\alpha$-tubulin acetylation in SMA patient cells and its effects on Golgi complex, since dispersed morphology was previously reported in SMN deficient cells.

**Method:** To evaluate the acetylation level of $\alpha$-tubulin, Western blot studies were performed using fibroblast cells of two clinically different SMA patients (type I and type II). Immunofluorescence stainings were performed to analyse acetylated microtubules as well as Golgi morphology, subsequently to the microtubule depolymerization and re-polymerization by nocodazole and a specific inhibitor of a major tubulin deacetylase, histone deacetylase 6 (HDAC6), treatments respectively.

**Results:** Western blot and immunofluorescence studies showed a significant reduction in acetylated $\alpha$-tubulin levels, especially in the perinuclear area of patient cells. Quantitative microscopic analysis demonstrated that dispersed Golgi morphology of SMA patient cells were restored by increasing $\alpha$-tubulin acetylation.

**Conclusion:** Our results showed that reduced $\alpha$-tubulin acetylation is associated with impaired Golgi morphology in SMA and increasing acetylation by HDAC6 inhibitors can restore both defects.

**P14**
## A Bioinformatic Approach For Determining Of Putative Phytoplasma Effector Proteins

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**Aim:** Phytoplasmas, which are plant pathogens belonging to the class Mollicutes, are transmitted by insects feeding on plant phloem. These pathogens manipulate host cell functions through the effector proteins they secreted and cause various anomalies in the plants. This ongoing study aims to identify effector proteins that cause damage in various agricultural crops. Due to the difficulty of establishing an axenic culture of these bacteria, culture-independent effector protein characterization is a crucial tool for phytoplasma research.

**Materials and methods:** First step, N-terminal signal peptides containing candidate effector proteins were identified by using SignalP 4.1, TMHMM v2.0 and Phobius tools. The results were compared with the signal peptides and genes organization of previously published effectors. To examine the relationship of the orthologs and to predict the function, Conserved Domain Database (CDD), PSI-BLAST, and HMMER tools used. Second step, candidate protein structures were predicted for structure/function analysis with the AlphaFold 2 tool, then investigated in Protein Data Bank (PDB) and AlphaFold Protein Structure Database by DALI server tool.

**Results:** As a result of our evaluations, one of the most potential effector candidates was SAP55 which has a metalloendopeptidase-like structure and contains the peptidase family M41 like domain. Interestingly, SAP55 does not have ATPase domain, but rather an additional $\alpha$-helix at its C-terminus.

**Conclusion:** As a result of our *in silico* study, an effector showing possible protease activity was discovered for the first time in Phytoplasmas. This study will provide great convenience for the wet lab part of our project.

## P15
## Quantum Mechancal Investigation Of Celecoxib And Arginine Interactions

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**Aim:** Celebrex is a subset of the non-steroidal anti-inflammatory drugs (NSAID), specifically for the COX-2 enzyme. Like other coxibs, it inhibits prostaglandin synthesis and thus reduces inflammation, which is a mediator of fever and pain. It is the only coxib currently available in the United States. Our aim is to investigate the interactions of celecoxib, Celebrex's active ingredient, with arginine present at the celecoxib-COX-2 interaction site to understand the binding mechanism.

**Method:** Firstly, conformational analysis of celecoxib was performed by using Spartan18 with molecular mechanics. By using the density functional theory (DFT) method for selected conformers, optimization and frequency calculation were carried out with Gaussian16 at $\omega$B97XD/6-311++G(d,p) level mimicking the solvent as water. Relative energies were used to select the most stable conformers for each molecule. Molecular structures were displayed using Discovery Studio Visualizer 2019 program. A similar workflow was applied for arginine. The interactions of the most stable celecoxib and arginine structures were investigated at the same DFT level using the structures formed using Spartan18. Non-covalent interactions, van der Waals, hydrogen bond, and steric hindrance, of structures, are obtained by using Multifwn 3.6 and VMD.

**Results:** The most stable conformers for celecoxib and arginine are calculated as 18, and 9, respectively. Using the most stable conformers, 33 dimer structures are generated. The optimization and frequency calculations are applied to determine the most stable interaction among these dimers.

**Conclusion:** The most stable structure is acquired between the hydrogen of celecoxib and the oxygen of arginine. The dominant non-covalent interaction is van der Waals. Steric effects and hydrogen bonds are also observed.

## P16
## Characterization Of Dynamic Recognition Nucleosome Core Particle Selecting Chromatin Factors

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**Background/aim**: The eukaryotic genome is organized by chromatin, which is a delicate ensemble of proteins and nucleic acids. The smallest unit of chromatin is the nucleosome core particle which is defined by two copies of four histones and approximately 145 base pairs of DNA. The nucleosome is an essential regulatory hub that interacts with transcription factors, chromatin remodelers, and other regulatory machineries. Arginine anchor motif is frequently encountered to bind a critical nucleosomal domain known as the acidic patch. We use molecular modeling and high-performance computing investigating common features of nucleosome recognition by five different chromatin factors with diverse functional roles albeit similar chromatin binding modalities.

**Materials and methods**: These chromatin-binding factors are: 1-53BP1, 2-SAGA DUB, 3-PRC1, 4-RCC1, and 5-Sir3. We use high-performance molecular dynamics simulations where we solvated each structure in a cubic water box in the presence of 161.5 mM NaCl and 5 mM Mg ions with applied CHARMM36m force field together with the OPC water model. We collected 100 ns long production trajectories using Gromacs software, version 2018, and analyzed them using in-house Python scripts, PyMOL and VMD.

**Results**: We find that all five chromatin factors exhibit varying levels of differences in the way they bind the two facades of the nucleosome core particle. These differences in the binding modes are also associated with the varying degrees of local stabilities of the chromatin factors whose primary epitopes involve flexible loop domains. We furthermore characterized the delicate interactions between the acidic patch and the arginine anchor motifs presented by each chromatin factor.

**Conclusion**: We used an *in silico* approach guided by detailed computational analyses to elucidate the major developments in biophysical analyses of the frameworks of chromatin-binding proteins with the nucleosome core particle, and reveal how these molecular machines access DNA to accomplish their various physiological roles.

## P17
## Criteria For The Assessment Of Workflow Management Systems For Bioinformatics

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**Aim**: Many scientific endeavors, such as molecular biology, have become dependent on large-scale data and its analysis. Workflow management systems (WFMS) enable the development of data analysis workflows (WF), their reproduction, and their application to datasets of the same type. However, there are far more than a hundred WFMS available to choose from, and no way to convert data analysis WFs among these. Therefore, the initial choice of a WFMS is important as it entails a lock-in to the system. Hence, fundamental criteria are needed to delineate among WFMS.

**Method:** By extracting such criteria from selected studies concerning WFMS and adding additional criteria, we arrived at five critical (reproducibility, reusability, FAIRness, versioning support, and security) and five important criteria (providing a graphical user interface, flexibility, scalability, shareability, and computational transparency) for the assessment of WFMS. We applied the criteria to the eight most cited WFMS in PubMed.

**Results:** Upon applying the five selected criteria to eight WFMS, we found that KNIME and Galaxy, although with limitations in versioning, are the only WFMSs supporting four of the chosen critical criteria at least in part. SnakeMake & Nextflow partially fulfill three criteria and GenePattern & Unipro UGENE somewhat fulfill two while Pegasus and CLC Bio only fulfill one criterion.

**Conclusion:** Currently, no WFMS supports any of the criteria in full and none fulfills the five critical criteria on a higher level. One criterion with little literature support that we included in the critical list is security. Security is especially important in medical data analysis, providing the basis for data protection and should therefore be fulfilled by a WFMS used in such an environment. We hope that suggesting these criteria will spark a discussion on what features are important for WFMS in scientific data analysis.

## P18
## Interaction Between Sars-CoV-2 Variants With Sus Scrofa Domesticus ACE-2

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**Aim**:To investigate the interaction between ACE-2 protein in Sus scrofa domesticus (farm pigs) with Sars-CoV-2 Spike variants such as Wuhan, Omicron, Delta and English using bioinformatic modelling.

**Methods**: We obtained nucleotides of Sus scrofa domesticus ACE-2 protein from BLAST, NCBI database. We detected two different profiles such as KX756982 ACE-2 protein and GQ262781 ACE-2 protein in Sus scrofa domesticus. Then, we translated the nucleotides into amino acids and checked for polymorphisms in MEGAX. We listed polymorphisms and detected silent and missensense mutations. We modelled 3d structure of ACE-2 protein by visualising in Phyre 2 and i-tasser with translated sequence. Then, we detected interaction surface for ACE-2 protein by comparing several variants using Chimera 1.14. We obtained data for RBD region of Delta, English, Wuhan, Omicron spike proteins from Protein Data Bank. Later, we docked ACE-2 proteins with RBD region of Delta, English, Wuhan, Omicron spike proteins by using HDOCK. We choose lowest docking score with correct binding region. Then we, visualize docking results with Pymol.
**Results**:RBD region of Wuhan, Delta, Omicron, spike proteins bind to Sus scorfa domesticus ACE-2 protein. Above, we showed HDOCK docking scores in the table.

|  | KX756982 ACE-2 protein | GQ262781 ACE-2 protein |
|---|---|---|
| **Wuhan** | -242.31 | -236.29 |
| **English** | -307.94 | -232.45 |
| **Delta** | -323.94 | -341.15 |
| **Omicron** | -311.31 | -320.56 |

A,C,E,G show Wuhan, English, Delta, Omicron respectively. (blue),Sus scrofa domesticus KX756982 ACE-2 (green). B,D,F,G show Wuhan, English, Delta, Omicron respectively. (blue), GQ262781 ACE-2 (green), RBD (red), ACE-2 interaction surface (purple)

**Conclusion**: Considering the above, it has been shown that Sars-CoV-2 Spike protein variants bind to ACE-2 proteins of Sus scrofa domesticus. The interaction surfaces of GQ262781 and KX756982 ACE-2 proteins attach to RBD region of Sars-CoV-2 spike variants with different docking scores. The mentioned protein-protein interactions, demonstrate that different types of variants binds to the surface with different docking scores.

**P19**
# Investigation Of Changes in Milk Proteome in Kefir Production

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**Aim**: Kefir is an important food source based in the Caucasus region. The beneficial effects of kefir on human health are emphasized in the literature. In this study, it is aimed to examine the changes in milk protein profiles during kefir production and storage processes by using mass spectrometry-based-omics approaches. In addition, the detection of kefir-based proteins that pass into milk from kefir microflora was also investigated within the scope of the study.

**Method**: During the production and storage processes of kefir, kefir samples were taken from the periods determined in the study, and milk proteins were extracted. Peptides were produced using Lys-C and trypsin enzymes using a classical proteomics approach. Fractionation of the peptides was then carried out. Peptide-containing samples were analyzed by nLC-QExactive-Plus mass spectrometry. The data obtained as a result of the analysis were processed with the Maxquant software and statistical analyses were performed. As a result of the analysis, the changes in milk proteins were shown. In addition, the proteins that pass into milk from kefir microflora and the functions of these proteins are shown in the study.

**Results**: According to the results obtained, significant changes were found in the milk proteome in 3 proteins between 0-12 hours, 39 proteins between 0-24 hours, 41 proteins between 0-7 days, and finally 42 proteins between 0-28 days. A significant change was observed in 23 proteins from the 24th hour. In the analysis, it was determined in the study that 398 kefir-based proteins were released into milk. 141 of these proteins could be quantified in the study by a label-free quantification approach.

**Conclusion**: In the study, changes in milk proteins during kefir production processes were determined. In addition, kefir-based proteins that pass into milk from kefir microflora were defined.

## P20
## Zymosan Enhances Anti-Tumoral Effects Of Radiotherapy In Breast Carcinoma

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**Aim:** Zymosan, which consists of beta glucan and mannan structures, stimulates innate immune response. Radiotherapy (RT) is widely used in cancer treatment and effectiveness of RT can be increased when combined with immunotherapeutic strategies. Up to our knowledge, there is no study examining the effect of zymosan co-treatment on anti-tumoral effects of RT in metastatic breast carcinoma model. Hence we here examined effects of zymosan alone and combined with RT on metastatic breast carcinoma in a syngeneic mouse model.

**Methods:** 4TLM s and 4TBM cells were originally derived from liver and brain metastases of 4T1 cells respectively. 4TBM and 4TLM cells were injected (100000 cells/0.1 ml) orthotopically into upper right mammary gland of 7-8 weeks old female Balb-c mice. Zymosan (5 mg/kg) was given i.p. for five times every other day starting from one day before RT. Zymosan is prepared as described before **[1].** RT was given locally at sub-optimal dose (6 Gray) 6 days after injection of tumor cells. Mice were sacrificed at the 26th day after injection of cancer cells. Tumor growth, lung metastasis and immune responses were evaluated.

**Results:** Zymosan did not alter proliferation of 4TLM and 4TBM cells in-vitro. Similarly, zymosan treatment alone did change primary tumor growth and the number of metastases. However, zymosan markedly increased the anti-tumoral and anti-metastatic effects of RT. In accordance zymosan markedly decrease baseline release of TNF-a while increasing IFN-g from peritoneal macrophages and mix leucocyte culture. Zymosan also decreased IL-6 release when combined with radiotherapy. Zymosan in combination with RT decreased tumor-infiltrating GR1+CD11b+ and increased CD3+CD4+ cells.

**Conclusions:** These results demonstrate that Zymosan enhances therapeutic effects of radiotherapy must likely by decreasing excessive inflammatory response and enhancing anti-tumoral immunity.

1. Daniel S. Martin et al. Cancer Res.1964 May;24:652-654.
2. Erin N et al. Breast Cancer Res Treat.2013 Jun;139(3):677-89.

## P21
## Investigation of Protein Denaturation Usıng Spectroscopc Methods

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**Aim**: Proteomics is comprehensive research of proteins that facilitates the development of novel biomarkers for diagnostic applications and exploration of prospective therapeutic targets. In bottom-up proteomics, also known as "shotgun", the peptide-centric approach aims to discover the identity and/or quantity of the proteins using unique peptide(s) via mass spectrometry(MS). Common steps in sample preparation are protein extraction, denaturation, reduction/alkylation, and enzymatic digestion, however, there is no standard protocol available.

**Method**: Here, the most common proteolytic digestion methods used in clinical studies were evaluated through spectroscopic methods. A reference protein, Bovine Serum Albumin (BSA) was dissolved in various buffers including ammonium bicarbonate (ABC), ABC-Urea, and Tris-HCl-Urea and treated with dithiothreitol (DTT) at 37□C and 60□ for 15 to 60 minutes. Circular Dichroism (CD) spectroscopy was used to monitor changes in protein secondary structure. Then, Ultraviolet-visible (UV-Vis) Spectroscopy was employed to investigate the change in signal associated with protein concentration before and after denaturation.

**Results**: According to the CD spectra taken at 60□C for 30 minutes, the most efficient denaturation buffer was ABC due to the greater negative signal decrease at 213 and 222 nm, characteristic of the $\alpha$-helical structure of BSA. For ABC-Urea and Tris-HCl-Urea buffers, there are variable results at 37□C and 60□C for 60 minutes in the CD spectrum. In the UV-Vis absorption spectra of BSA in ABC buffer, the signal change for total protein denaturation was observed clearly at 280 nm.

**Conclusion**: Results suggest that protein denaturation is very sensitive to buffers and experimental conditions. We further investigated the quantitative relationship between protein concentration and corresponding unique peptide's responses. Results showed that unique peptides' responses were variable and not correlated with corresponding protein concentrations. Future studies should focus on other steps involved in proteolytic digestion to evaluate procedures and standardize protocols for efficient and reproducible analysis.

## P22
## Evaluation Of Peptide Of OipA Gene Gastrit Tissues By MALDI-Imaging

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**Objective:** Outer membrane virulence factors are important for Helicobacter Pylori(H.pylori) to attach to the gastric epithelial surface and cause infection.Outer membrane inflammatory protein A (OipA) H.pylori's attachment to the gastric epithelial surface and its involvement in inflammation have been demonstrated by studies on ulcers,gastritis,gastric cancer.Investigation of protein profiles caused by H.pylori, which is the cause of infections, is important because these proteins have the potential to be biomarkers that can be used to diagnose the infection and determine its prognosis.Matrix-assisted laser desorption/ionization(MALDI)-Imaging mass spectrometry combines histological information with high resolution and accuracy and determines the spatial distributions of analytes on tissue.In this study, it is aimed to analyze peptide profiles of gastritis tissues in which H.pylori OipA gene is on/off by MALDI-Imaging mass spectrometry.

**Materials and Methods:** Sections of 3µm thickness from FFPE gastritis tissues were taken on indium-tin-oxide coated slides in microtome.After washing with xylene and decreasing alcohol concentrations, antigen retrieval was performed with 10mM citrate buffer solution.100ng/µl trypsin enzyme was used to digestion proteins into peptides, and then tissue was coated with 7mg/ml $\alpha$-CHCA matrix prepared in 70%acetonitrile, 1%trifluoroaceticacid.Tissues were analyzed with RapifleX-MALDI-Tissue-Typer.The number of monoisotopic peptides, peptide localizations associated with histological and hierarchical clustering analyzes were used to evaluate the results.

**Results:** The peptides obtained as a result of the analysis of gastritis tissues in which the H.pylori OipA gene on/off were evaluated in terms of peak numbers and signal intensities.The highest number of peptides were obtained from tissues with H.pylori OipA gene on group.However, S/N ratios and relative intensity values of 5 peptides with the highest signal intensity were higher in tissues with the H.pylori OipA gene on group.Peptide identifications were made by LC-MS/MS method,382 proteins were identified.

**Conclusion:**The localization and identification of the peptides of gastritis tissues with on/off OipA gene in accordance with histology have been completed.

## P23
## Investigation Of The Proteins of Leishmania Tropica Causing Viscerotropism

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**Objectives**: Leishmania is a kind of parasite which causes a disease called leishmaniasis. The main clinical forms are Cutaneous (CL) and Visceral Leishmaniasis (VL). Genetic exchange between different Leishmania types has been reported; *L. tropica* can show both cutaneous and visceral symptoms. *L. tropica* isolates from CL and VL patients are analyzed with mass spectrometric shotgun proteomics to compare the protein expressions and determine differential proteins that might cause viscerotropism in *L. tropica*.

**Methods**: Proteins were purified with acetone precipitation. Total protein quantification is determined by Bradford method before MS/MS analyses. Filter-aided sample preparation method and in-solution digestion procedures were applied.
Peptides were fractionated by concatenation to reduce the complexity and improve the proteome coverage. The HPLC system was SHIMADZU Prominence UFLC. For LC-MS/MS analysis, DIONEX UltiMate 3000 was used.
Data obtained from LC-MS/MS analyses were searched in the database by the Mascot server. Common and differential proteins between CL and VL samples were determined.

**Results**: The spectral counting data (emPAI) represents protein abundance in samples. T-test (p=0.05) determined that 91 of the 2102 proteins showed statistically significant changes between cutaneous and visceral isolates.
Down-regulation of immunogens, including paraflagellar rod proteins, EF-1$\alpha$, and surface antigens in visceral group might aid in hiding from macrophages or reducing the immune system stimulation by expressing these proteins in a lesser amount. Also, peroxidoxins and cytochrome b5 might provide resistance to oxidative stress induced by macrophages and contribute the parasite survival. Finally, up-regulation of endoribonuclease in visceral isolates might protect the parasite against the accumulation of damaged proteins by ROS or any other sources of stress.

**Conclusions**: *L. tropica* might reduce essential functions and express minimum variety of proteins to avoid immune system recognition. Moreover, there were statistically significant proteins playing essential roles in *L. tropica* survival in macrophages in case of oxidative stress.

## P24
## Analysis Of Protein Behavior During Enzymatic Attack With Mid-Infrared Spectroscopy

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**Aim:** Exploring the dynamical properties of biological samples relying on physical methods is crucial to solve cutting-edge problems. Herein, we aimed to analyze behavior of β-lactoglobulin on the molecular level in response to enzymatic attack at 25°C/45°C after isotopic exchange by using vibrational infrared (IR) spectroscopy. Enzymatic hydrolysis of peptide bonds in the polypeptide chain, proteolysis, is widely used in proteomics and industry. Proteolysis causes cleavage of proteins into smaller pieces by proteases.

**Materials and Methods:** After isotopic exchange of samples from hydrogen to deuterium, the enzymatic reaction was initiated in deuterium oxide ($D_2O/^2H_2O$) buffering conditions. A whey protein β-lactoglobulin was cleaved by trypsin. Substrate-to-enzyme ratio was adjusted to 5000:1. Blank protein sample and reacted samples were measured both at 25°C and 45°C for 3h by using Fourier Transform-infrared spectrometer (Perkin Elmer UATR Two). After the samples were sandwiched between demountable $CaF_2$ windows, their spectra were recorded in transmission mode, by controlling the temperature with circulating water bath. Percentages of secondary structures were calculated with OPUS program.

**Results:** Secondary structural alterations in β-lactoglobulin due to enzymatic attack at 25°C and 45°C were compared. The IR signal absorbing at 1644 cm⁻¹ (random coils) increases at 25°C while α-helices and β-sheets undergo slight alterations during digestion. However, the IR signals absorbing at 1650/1541 cm⁻¹ (α-helix) and 1681/1633 cm⁻¹ (β-sheet) diminish at 45°C due to trypsin when compared to the blank sample. Besides, the IR signals absorbing around 1605-1580 cm⁻¹ (free carboxylates/products) increase upon tryptic digestion (largely at 45°C).

**Conclusion:** This work provides tracking of protein dynamics and analysis of globular protein behavior during enzymatic attack by using mid-IR spectroscopy in $D_2O$ buffering system at various temperatures. Proteolysis mechanism will be further investigated with biophysical/physicochemical methodologies. This work was funded through international TUBİTAK-2532 Russia Bilateral Cooperation project (Grant#119N423 to G. GÜLER).

## P25
## Developing Of New Data Processing Software In Mass Spectrometry-Based Imaging

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Mass spectrometry is one of the most important techniques used in the comprehensive analysis of biomolecules. In the thesis, an open-source and free-to-use interface has been developed on the python platform for MALDI imaging data. It has been accepted that the developed interface has a design for users to use it easily. The interface has been developed in accordance with the processing of ".imzML" files, which is a widely used data format in mass spectrometry-based imaging. The spectrum function developed for MALDI imaging data allows us to see the maximum peak intensities by plotting the spectrum graph. The developed interface provides the basis for creating an image by reading the value from the graph for a single mass/charge. With this feature, the user can determine the desired mass/charge (m/z) value using these graphic tools. It also provides the opportunity to examine the formed isotope distributions clearly. Visualization functions in the developed interface are created using the pyimzML library; they are realized by considering the m/z value range determined depending on the tolerance. The resulting bioimage is colored with a heat map. Assistance in data interpretation is provided with statistical tools. The developed software can process the data of MALDI-TOF and DESI (desorption electrospray ionization) devices and visualize their images. Among other features, it allows the user to change color maps, enter multiple m/z and tolerance values into the interface, and see different density images simultaneously. Using the interface developed in this thesis, the outputs obtained after data processing were also compared with commercial and open-source software. As a result, an open-source, user-friendly interface that can handle common mass spectrometry-based data types has been developed in the thesis. The developed interface is made available to users on the GitHub page (https://github.com/betulkarabudak).

## P26
## Quantification Of DNA Repair Protein HPARP1 In Leukocyte By LC/ID-HR-MS

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**AIM**: Poly-ADP-Ribose-Polymerase-1 (PARP1) is an essential component of base-excision-repair (BER) involved in the repair of oxidative DNA damage. PARP1 can directly repair DNA strand breaks and, it is known to be involved in many other cellular processes. Quantification of PARP1 in human leukocyte might represent predictive and prognostic value since its expression is found to be altered in cancer tissues and PARP1-inhibitory drugs are used in the treatment of cancer. However, the hPARP1 protein levels in leukocytes are quantified by indirect methods, yet there is no such study that shows the accurate quantification of hPARP1 in leukocytes. This study shows the direct measurement of hPARP1 in leukocytes by liquid chromatography-isotope dilution-high resolution-mass spectrometry (LC-ID-HR-MS) with targeted proteomics approach.

**Method**: Blood samples were collected then leukocytes were isolated. Total protein from leukocytes was obtained then analysed on LC-ID-HR-MS through the "bottom-up" proteomics approach. Lys-13C6, 15N2- or Arg-13C6, 15N4-labelled tryptic peptides of hPARP1 were used for the quantitative measurements.

**Results**: Endogenous and stable isotope labelled peptide couples of hPARP1 protein provided quantitation with high sensitivity. Four PARP1 tryptic peptides were detected and as calculated on SwissProt detection of four tryptic peptides were enough for the quantification of hPARP1 level (with $p < 0.05$). hPARP1 protein was obtained at ng/mg protein level in leukocyte.

**Conclusion**: The absolute quantification of hPARP1 from whole blood can contribute to the clinical and basic research studies in terms of prediction and prognosis. Along with hPARP1 protein, other DNA repair proteins which are also involved in BER pathway, APE1, NEIL1, NTHL1, OGG1, and Polb are found to be detectable with the proposed method. With the absolute quantification of these DNA repair proteins, the nature of DNA repair can be explained further and prognostic value of these quantification from whole blood samples may help to act against the diseases.
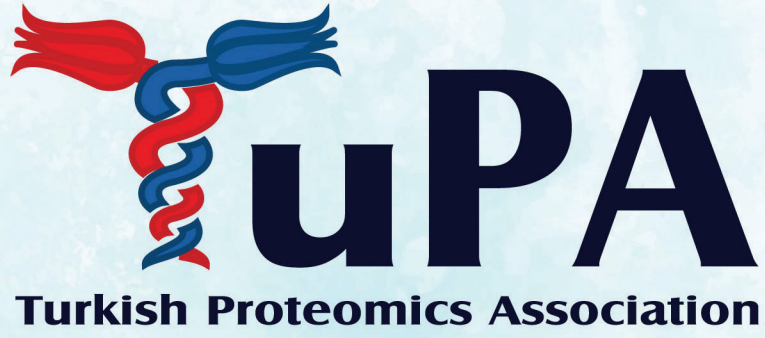
## P27
## Rapid, High-resolution Ambient Temperature Structure Determination at Turkish Delight

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X-ray crystallography is a powerful and robust structural biology technique. This technique unravels high-resolution atomic structures and elucidates mechanistic details of enzyme catalysis which is critical to understand protein function and dynamics. Our group recently initiated a Turkish Structural Biology Consortium and was part of establishing a state-of-the-art home X-ray diffractometer known as Turkish Light Source (Turkish DeLight) at the University of Health Sciences. Turkish DeLight, in addition to being capable of performing single-crystal cryo-crystallography, also enables us to determine high-resolution biomacromolecular structures at ambient temperature. Thanks to our customized XtalCheck-S plate reader system implemented in our beyond-the-state-of-the-art home X-ray source, we can obtain diffraction data from biomacromolecules in a significantly short time with mitigated radiation damage. In our work, we used chicken egg lysozyme as a model protein and determined its structure to 2.39 Å resolution at ambient temperature using Rigaku's XtaLAB Synergy Flow System XRD equipped with a modified XtalCheck-S Terasaki plate reader adaptor. We compared the ambient temperature structure of the lysozyme with our previously published cryogenic structure of the same protein at 1.7 Å resolution (PDB: 7Y6A). Here we provide a paradigm-changing example of a high-resolution protein crystal structure obtained from a home X-ray source "Turkish DeLight" at near-physiological temperature by switching to a "Warm Turkish DeLight" mode. The main purpose of this experimental setup is to "serially" collect preliminary diffraction data from protein crystals at ambient temperature using a multiwell-multicrystal plate reader as an alternative to serial femtosecond and millisecond X-ray crystallography (SFX/SMX) techniques performed at XFELs and synchrotrons respectively.

TuPA

Turkish Proteomics Association