STRUCTURAL PROTEOMICS OF FTSH COMPLEXES USING CROSS-LINKING MASS SPECTROMETRY

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ABSTRACT

STRUCTURAL PROTEOMICS OF FTSH COMPLEXES USING CROSS-LINKING MASS SPECTROMETRY

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FtsH proteases are responsible for membrane protein quality control and are highly conserved in bacteria, mitochondria, and chloroplasts. FtsH contains an ATP-dependent endonuclease that degrades improperly folded integral membrane proteins and interacts with other membrane proteins HflK and HflC, modulating this process. This thesis investigated the composition and structure of FtsH complexes using cross-linking mass spectrometry (XL-MS) based structural proteomics. Recombinant expression of protein complexes was performed in *E. coli*, inner membranes were solubilized, and proteins were purified using affinity chromatography. The residues of proteins in close proximity were covalently bound to each other via cross-linkers. Cross-linked proteins were then enzymatically digested and analyzed by the high-resolution MS. Commonly used XL-MS software tools, MaxLynx, MeroX, MS Annika, and XlinkX were assessed to elucidate the protein interactions within the membrane protein complexes containing FtsH, HflK, and HflC. The cross-link search analysis yielded over 300 inter- and intra-protein interactions. The results provide valuable information regarding the complex structure and function and could be applied to molecular docking for structural modeling.

Keywords: Cross-linking Mass Spectrometry, Protein Interactions, Structural Proteomics, FtsH Complexes, Membrane Proteins

ÖZ

ÇAPRAZ BAĞLAMA KÜTLE SPEKTROMETRESİ KULLANILARAK FTSH KOMPLEKSLERİNİN YAPISAL PROTEOMİĞİ

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FtsH proteazları, membran protein kalite kontrolünden sorumludur ve bakteri, mitokondri ve kloroplastlarda yüksek oranda korunur. FtsH, yanlış katlanmış integral membran proteinlerini parçalayan ATP'ye bağlı bir endonükleaz içerir ve bu işlemi modüle eden diğer membran proteinleri HflK ve HflC ile etkileşime girer. Bu tez, XL-MS tabanlı yapısal proteomik yaklaşımını kullanarak FtsH komplekslerinin bileşimini ve yapısını araştırdı. Protein komplekslerinin rekombinant ifadesi *E. coli*'de gerçekleştirildi, iç membranlar çözündürüldü ve proteinler afinite kromatografisi kullanılarak saflaştırıldı. Protein komplekslerinin yakın mesafedeki amino asitleri, çapraz bağlayıcılar aracılığıyla birbirlerine kovalent olarak bağlandı. Çapraz bağlı proteinler daha sonra enzimatik olarak sindirildi ve yüksek çözünürlüklü MS ile analiz edildi. Yaygın olarak kullanılan XL-MS yazılım araçları, MaxLynx, MeroX, MS Annika ve XlinkX, FtsH, HflK ve HflC içeren membran protein kompleksleri içindeki protein etkileşimlerini aydınlatmak amacıyla değerlendirildi. Çapraz bağlantı arama analizi, 300'den fazla proteinler arası ve protein içi etkileşim ortaya çıkardı. Sonuçlar, kompleks yapısı ve işleviyle ilgili değerli bilgiler sunuyor ve yapısal modelleme için moleküler yanaştırma metoduna uygulanabilir.

Anahtar Kelimeler: Çapraz Bağlı Kütle Spektrometre, Protein Etkileşimleri, Yapısal Proteomiks, FtsH Kompleksleri, Membran Proteinleri

To my dearest family, late father and friends

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LIST OF ABBREVIATIONS

Transmembrane Domain (TD)

Protein Quality Control (PQC)

Proton Motive Force (PMF)

Endoplasmic Reticulum (ER)

Inner Membrane Proteins (IMP)

Mass Spectrometry (MS)

Ion Exchange Chromatography (IC)

Size Exclusion Chromatography (SEC)

Affinity Chromatography (AC)

Glutathione-S-transferase (GST)

Cross-linking Mass Spectrometry (XL-MS)

Tandem Affinity Purification (TAP)

Cryogenic Electron Microscopy (Cryo-EM)

Mass-to-charge (m/z)

Protein Data Bank (PDB)

Electrospray Ionization (ESI)

Matrix-Assisted Laser Desorption Ionization (MALDI)

Time-of-flight (TOF)

Tandem mass spectrometry (MS/MS)

Collision-Induced Dissociation (CID)

High-energy Collisional Dissociation (HCD)

Hydrogen–Deuterium Exchange Mass Spectrometry (HDX-MS)

n-Dodecyl-B-D-Maltoside (DDM)

Phenylmethylsulfonyl Fluoride (PMSF)

Disuccinimidyl Dibutyric Urea (DSBU)

Bis(sulfosuccinimidyl)suberate (BS3)

Dimethyl Sulfoxide (DMSO)

Dithiothreitol (DTT)

Iodoacetamide (IAA)

High-Pressure Liquid Chromatography (HPLC)

Gene Ontology (GO)

Protein-Protein Interaction (PPI)

Solubilized Membrane (SM)

Purified Protein (PP)

Total Ion Chromatogram (TIC)

Small Angle X-ray Scattering (SAXS)

Graphical User Interface (GUI)

CHAPTER 1

INTRODUCTION

1.1 Membrane Proteins

Membrane proteins are proteins that are embedded into the lipid bilayer of the cell membrane, forming a barrier between the cell and the external environment. They consist of amino acids which fold as a hydrophobic surface contacting with the alkane chains of the lipids and hydrophilic surface contacting with the aqueous phases on the sides of the membrane and hydrophilic polar head groups of the lipids in the bilayer.[1]

Membrane proteins play crucial roles in cells, such as cell trafficking, signal transduction, ion transport, cell-cell interactions. They make up ~30% of the cell proteome and their disruption leads to various diseases, such as cystic fibrosis, retinitis pigmentosa, and other numerous heritable diseases.[2] Therefore, they are studied as a target for developing new therapeutics.[3,4]

There are two classes of membrane proteins: integral membrane proteins and peripheral membrane proteins. These classes are illustrated in **Figure 1.1a**. Integral membrane proteins span the lipid bilayer of the membrane and are stabilized by hydrophobic interactions of the transmembrane domains (TD) and lipids. Two types of integral membrane proteins are present: alpha-helical and beta-barrels, as demonstrated in **Figure 1.2a**. Alpha-helical integral membrane proteins are the most abundant ones and are encoded in most organisms, whereas beta-barrel integral membrane proteins are found in the outer membranes of bacteria, mitochondria, and chloroplasts.[5,6] Peripheral membrane proteins, on the other hand, are amphipathic proteins interacting with the cell membrane directly or indirectly. They do not cover

the entire lipid bilayer; however, could be anchored into the lipid bilayer with a hydrophobic domain.[7]

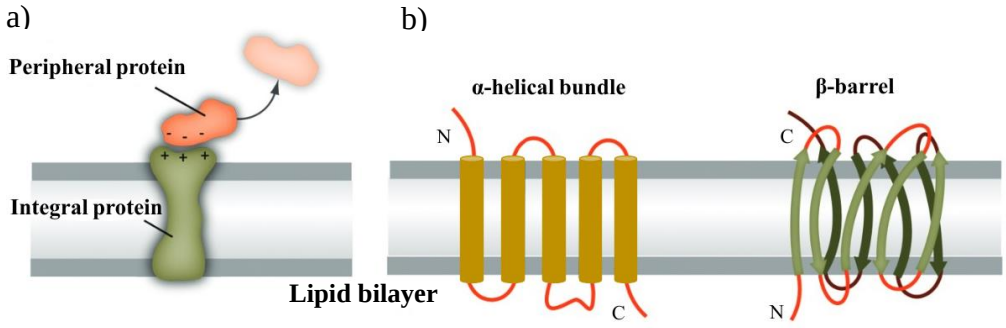


Figure 1.1. a) Classes of membrane proteins and b) types of integral membrane proteins regenerated from "An Introduction to Membrane Proteins" by Hedin et. al. J. Proteome Res. 2011, 10 (8), 3324–3331.

1.1.1 Quality Control Mechanism of Membrane Proteins

Cell membrane comprises a considerable number of proteins and they have various important functions for the cells. Quality control is highly important for the cells. They go through different quality control mechanisms for their proper folding, insertion, stability, and function.[8]

After translation, some part of nascent chains of proteins undergo degradation due to mistakes in biogenesis or maturation. Errors in the protein synthesis cause severe cellular damage and toxicity, and leads to diseases and many pathologies.[9] In the case of misfolding and/or aggregation, these proteins are either degraded by proteases, or they are promoted for proper folding by chaperones.[10]

Chaperones target the unfolded and partially folded polypeptide chains, having hydrophobic regions exposed on the surface of the proteins. These proteins repeatedly bind to the chaperones and are released from them.[11]

In the case of protease degradation, there are different compartments for the action of proteases of the protein quality control system. Proteins to be degraded are first unfolded and then transferred to the proteolytic cavities of the proteases to be broken down into small peptides. Cytosolic proteins are targeted for destruction by ubiquitination machinery.[12] In Golgi complex and Endoplasmic Reticulum (ER) different degradation machinery are triggered upon the misfolding.[13] The functioning of the protein quality systems is simply represented in **Figure 1.2**.

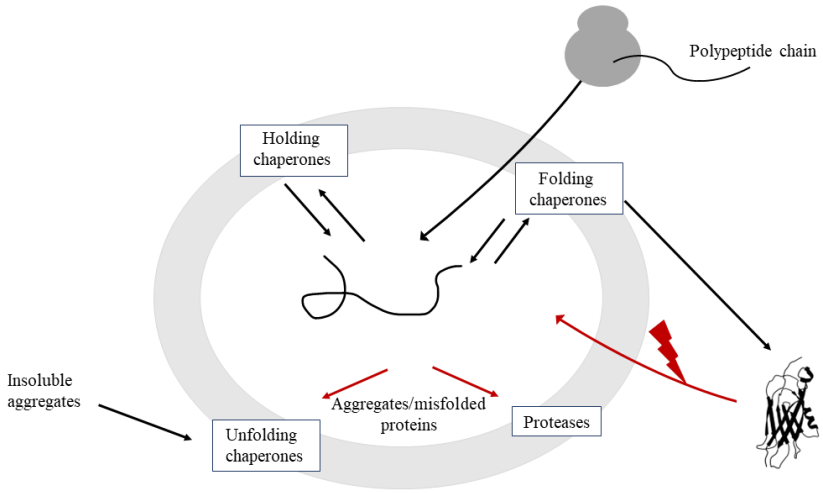


Figure 1.2. The representation of the functioning of protein quality control (PQC) systems. The figure is regenerated from "Protein Misfolding and Human Disease" by Gregersen et. al. Annu. Rev. Genomics Hum. Genet. 2006, 7, 103–124.

1.1.1.1 The Role of FtsH Complexes in Bacterial Membrane Quality Control

As for the bacterial membrane protein quality control, SecYEG-SecDF-YajC-YidC holo-translocon is in action in the inner membranes of *E. coli*. Seven membrane protein subunits, including SecYEG responsible for protein secretion and YidC for membrane protein insertion, are present in this complex. Transmembrane proton-motive force (PMF) assists the protein translocation by the sub-complex of SecD and

SecF through SecYEG.[14] FtsH protein is a member of AAA+ protease family, and it degrades the misassembled inner membrane proteins (IMPs) contributing to the bacterial membrane protein quality control.[15] FtsH is conserved in bacteria, mitochondria and chloroplast.[16] The ligand interaction sites of FtsH is illustrated in **Appendix**. *E. coli* FtsH consists of a periplasmic domain at the N-terminus and a cytoplasmic domain with ATPase and zinc-protease domains at the C-terminus. C-terminal protease cleaves the unfolded proteins with Zn^{+2}.[17] FtsH is known to form a very large membrane-bound complex with HflK and HflC, comprising periplasmic domains. HflKC modulates the proteolytic activity of FtsH and the FtsH-HflK-HflC complex plays a chaperone role to stabilize proteins in mitochondria, and a modulator role to limit ATPase activity of FtsH.[16,18,19] Information of FtsH, HflK, HflC is presented in **Table 1.1**.

Table 1.1. The information of FtsH, HflK, and HflC proteins.

	FtsH	**HflK**	**HflC**
UniProt ID	P0AAI3	P0ABC7	P0ABC3
Length (Amino Acids)	644	419	334
Molecular Weight (Da)	70,708	45,545	37,650
Structure	7WI3	7WI3	7WI3

1.2 Protein Complexes

Polypeptide chains assemble by non-covalent protein-protein interactions forming protein complexes. They are the crucial functional units involved in various biological processes and functioning within the cell. They provide valuable insight into the behavior of biological systems. When a protein complex forms, it could activate and/or inhibit other proteins and/or protein complexes, and that way they lead to different functions. Protein complexes are known to take part in important processes, such as transcription, translation, folding, quality control, and signal transduction, in cell systems.[20]

A protein complex could catalyze multiple reactions simultaneously or sequentially, and complex cellular processes start taking place. Metabolic and cellular pathways, signal transduction pathways, and other functions in the cells are regulated by the enzymatic activities of protein complexes. Enzymatic activities of the protein complexes catalyze different biochemical reactions that are important for the cells. These reactions are diverse, essential, and vital for the integrity, functioning, interaction, and survival of the cells.[21] Any defect, mutation or aggregation occurring to proteins, may result in various diseases and protein complexes aid in the understanding and explaining these processes.[22] Understanding of protein complex structure, dynamics and function has accelerated drug design and discovery research.[23]

1.3 Structural Proteomics

Determination of the three-dimensional structures of the proteins and protein complexes are crucial for enlightening the functions. Moreover, it facilitates the identification of protein-protein interactions and pathways they are included in. Structural proteomics is a field of study where invaluable insights into three-dimensional structures of the proteins are obtained, assisted by mass spectrometry

(MS). It is used to understand the relationship between protein sequence, structure, and function.[24] In recent years, structural proteomics, along with main structural biology methods, has broadened our knowledge and perspective of structures of proteins and protein complexes.[25] Structural proteomics has provided us with an essential gate for the discovery of proteins and/or protein complexes that are important in drug targets and protein-ligand interactions.[26] The studies of structural proteomics involve two important steps: protein expression and/or purification, and structural analysis of proteins.

1.3.1 Sample Preparation for Structural Proteomics: Protein Expression and Purification

The first stage of the structural determination of the proteins and protein complexes of interest is the expression of them in the desired system and/or purification with an appropriate method.

- **Recombinant Protein Expression**

In order to express target proteins, DNA templates of genes of interest are easily constructed through recombinant DNA techniques. Recent recombinant protein expression techniques consist of transforming/transfecting cells with a vector which has the template and culturing the cells. Thus, these cells first transcribe DNA into mRNA and then translate the protein of interest. Before the purification process, the cells are lysed for the extraction of the expressed proteins. According to the type of protein of interest different protein expression systems are preferred. Mammalian, insect, yeast, bacterial, algal, and cell-free systems are available for recombinant protein expression.[27]

- **Protein Purification**

Protein purification is the process of isolation of proteins from the complex mixtures of cells, tissues or organisms. The desired protein/protein complex is separated from other proteins and matrices. Proteins are isolated through various physical and biochemical approaches. Protein purification comprises the combination of following steps: extraction, precipitation, differential solubilization, ultracentrifugation, and chromatographic methods. After the cells or tissues are broken and proteins are extracted, a centrifugation process is applied for the isolation of proteins. Chromatographic methods, such as ion exchange chromatography (IC), size exclusion chromatography (SEC), affinity chromatography (AC), and immunoaffinity chromatography, are most commonly used methods to isolate the desired protein.

In affinity chromatography, different affinity tags, such as strep-tag and glutathione-S-transferase (GST), are used for protein purification in which recombinant proteins with an engineered specific peptide or a tag are purified. This process is effective in terms of improving the yield, purity, and solubility of the target protein.[28] The pull-down methods are used for the purification of protein complexes. Physical interactions of the proteins with each other, in other words, protein-protein interaction networks, are obtained through pull-down assays. Affinity pull-down, tandem affinity purification (TAP), and co-immunoprecipitation are the methods included. In affinity pull-down and TAP, affinity tags are used, whereas antibodies are used against target protein in co-immunoprecipitation method.[29] The processes of protein expression and purification play a vital role in determining the structure, function, and interactions of the protein of interest. Protein samples used for the structure determination should be as pure, homogeneous, and highly concentrated as possible.

1.3.2 Structural Analysis of Proteins and Protein Complexes

Several methods emerged for the structural elucidation of proteins and protein complexes and determination of protein-protein interactions, such as X-ray crystallography, cryogenic electron microscopy (cryo-EM), and mass spectrometry (MS).

X-ray crystallography provides the structural information by obtaining the arrangement of atoms in a protein of a crystal exposed to X-rays diffracting into specific directions. Cryo-EM is electron microscopy carried out on cryogenically frozen samples. Proteins are bombarded with electrons and images of individual molecules are obtained. On the other hand, mass spectrometry is an analytical method measuring the mass-to-charge ratio (m/z) of ions. It allows the qualitative and quantitative analysis of proteins. Each method has its own advantages and disadvantages. They could be integrated into each other in order to obtain accurate, reliable and high-resolution structures, which is known as "integrative structural biology".

1.3.2.1 X-ray Crystallography

X-ray crystallography is one of the most reliable, precise, and powerful methods used in structural biology and most of the structures in the Protein Data Bank (PDB)[30] have been determined by X-ray crystallography. It is a method used to acquire a three-dimensional structural information from a protein crystal. In the crystal structure determination workflow, first, a protein of interest is purified and crystallized. The crystallization process is applied to highly pure samples by subjecting them to different crystallization conditions and precipitants. The crystal is then subjected to the beam of X-rays. The protein crystal diffracts the X-ray beams into different, unique patterns before being analyzed to a map of the distribution of electrons in the protein. The location of atoms is determined through the electron

density map. Detailed atomic information regarding even the complex biomacromolecules incorporated into the crystal could be obtained through X-ray crystallography.[31] However, the limitations of X-ray crystallography in terms of the sample preparation and capturing the dynamics remain challenging in this method.[32] Membrane proteins are difficult to crystallize and efforts are being made to improve the challenges of obtaining pure protein crystals.[33]

1.3.2.2 Cryogenic Electron Microscopy (Cryo-EM)

Cryo-EM is a powerful technique used to observe the conformations of biomacromolecules directly in their nearly native environments. It provides a detailed insight into the dynamic behaviors of the molecules.[34] The protein of interest is flash-frozen and then bombarded with electrons producing microscope images of each molecule. These images (micrographs) are used to reassemble the three-dimensional structure or shapes.[35] Unlike X-ray crystallography, Cryo-EM technique does not require crystals. It requires a much smaller sample amount (between 50 nm and 5 μM) and large variety of sample type can be used for analysis.[34,36] High-resolution structures are desired to see every atom in the electron density map and nowadays, high resolution structures, even less than 2 ångströms (Å), can be obtained through this method.[37,38] However, the samples used for Cryo-EM technique should be stable, pure and homogeneous and it is challenging to analyze small proteins and protein complexes (less than 65 kDa) with this methods.[39]

1.3.2.3 Mass Spectrometry for Proteomics

Mass spectrometry is an analytical method measuring the mass-to-charge ratio (m/z) of molecules. It is a sensitive, selective, versatile, and high-throughput tool

applicable to all types of samples. The basic principle of mass spectrometry includes the first ionization step where the target molecules are converted into gas-phase ions. The formed ionic species are then separated and analyzed based on their m/z. As a final step, detectors measure the ion current and create a mass spectrum.

MS-based proteomics includes a wide range of studies from the sequences of proteins to the three-dimensional structures qualitatively and quantitatively.[40] Bottom-up approach, which is peptide centric, is the most common approach in MS-based proteomics. The conventional workflow includes the extraction of proteins in suitable buffers, denaturation of proteins in the samples by dithiothreitol (DTT), alkylation by iodoacetamide (IAA), and digestion by trypsin which has specificity on lysine and arginine side chains. The resulting peptides are separated by chromatography and analyzed by high-resolution mass spectrometry which will be explained in detail below. The protein search software, such as MaxQuant[41,42], Mascot[43] and Proteome Discoverer[44], first identify the precursor ions (MS1) and verify the peptides searching the tandem MS spectra through libraries and generate a protein list. Thus, the proteins in the samples are detected and quantified. Furthermore, biological insights where proteins are involved could be obtained.

A mass spectrometer has essential components: Inlet system, ion source/ionization system, mass analyzer, detector, data system, vacuum system, and electronics. These components are illustrated in **Figure 1.3**. First, the sample is transferred into the ion source via the inlet system. An ion source is required to convert the neutral molecules into charged molecules. Electrospray Ionization (ESI) and MALDI (Matrix-assisted laser desorption ionization) are two soft ionization techniques which allow the analysis of larger and non-volatile biomolecules such as proteins and peptides.[45] Analyte ions are separated and analyzed in a mass analyzer according to their mass. Ion motions are controlled by applying magnetic or electric fields. Time-of-flight (TOF) and the novel technology Orbitrap, which offers high resolving power, mass accuracy, and dynamic range[46], are the most common mass analyzers used for the analysis of proteins.[40] Orbitrap is based on the electrostatic trapping of ions. The ions

are collected from the linear trap quadrupole by C-trap and injected into the orbitrap. They are kept in oscillating movement by the electrical field applied.

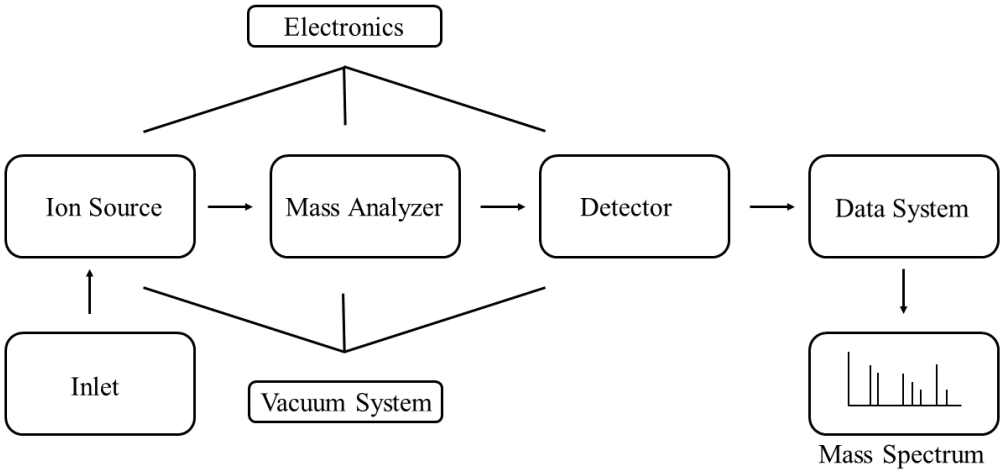


Figure 1.3. Basic components of a mass spectrometer.

Resolution is one of the main performance metrics provided by a mass analyzer.[47] The ability of the instrument to differentiate between masses is resolving power and it's given as the ratio of the mass of the first peak (m) over the mass difference between two adjacent peaks resolved (Δm):

$$\text{Resolving Power (RP)} = \frac{m}{\Delta m}$$

Low mass resolution leads to limited mass accuracy; therefore, the false discoveries increase in the protein identification. High mass resolution is desired from the mass spectrometer for accurate mass measurements, resolving every species, increasing the accuracy of the quantification. The Orbitrap mass analyzer is nowadays one of the most popular technologies in MS applications.[48] Ultra-high resolving power over 1,000,000 with Orbitrap technology is now reached.[49] This technology is recently

preferred to be used in structural proteomics approaches where cross-linking mass spectrometry is used.[50–52]

High mass resolution, additionally, allows for the selection of precursor ions in tandem mass spectrometry for structural analysis. Different mass analyzers are combined creating hybrid instruments to increase the mass resolution, mass accuracy, sensitivity, and at the end, capability of the analysis. Tandem mass spectrometry (MS/MS) is a technique where two or more mass analyzers are coupled to characterize and verify structural composition. The schematic of the technique is illustrated in **Figure 1.4**. Ions with particular m/z (precursor ions) are selected and fragmented by fragmentation techniques, such as collision-induced dissociation (CID) and high-energy collisional dissociation (HCD), creating product ions. In CID, ions are activated through collisions with neutral gas molecules (helium, nitrogen, argon) and the kinetic energy is converted to internal energy. This energy leads to bond breakage and fragmentation. HCD is a CID technique that is associated with Orbitrap instruments. The kinetic energy of the precursor ion is increased through voltage offsets and the ions collide with nitrogen molecules. They are transferred back to C-trap and injected to Orbitrap mass analyzer.[53]

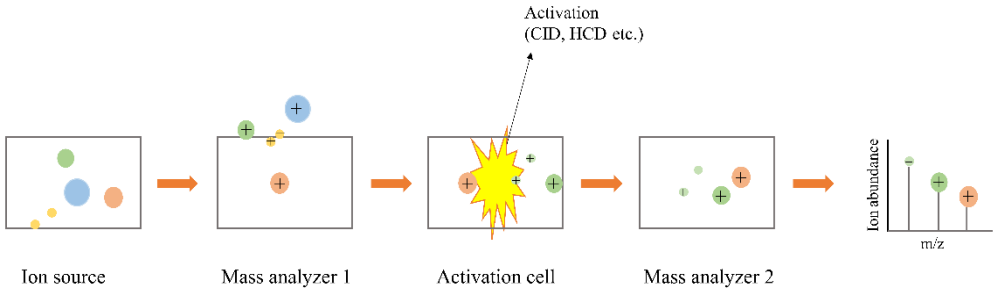


Figure 1.4. Schematic diagram of the functioning of tandem mass spectrometry technique.

- **Importance of Mass Spectrometry Method in Structural Proteomics**

The analysis of protein structure with mass spectrometry is an emerging method compared to conventional structure determination techniques mentioned above. Mass spectrometry offers several advantages: 1) MS is not restricted by sample preparation requirements. 2) It is compatible with low abundant analytes and complex samples; therefore, it can be applied to a wide range of samples, including organelles, cells, and even tissues.[54] 3) Proteins and protein complexes exhibit dynamic behavior on distinct time scales, thus it is difficult to capture the transitions and states.[55] Mass spectrometry is, therefore, advantageous for selectively analyzing complex samples containing different protein forms.

Recently, multiple analytical approaches based on mass spectrometry have been derived, such as native mass spectrometry, hydrogen–deuterium exchange mass spectrometry (HDX-MS), and cross-linking mass spectrometry (XL-MS). Native mass spectrometry is a method enabling the analysis and characterization of intact proteins and protein complexes.[56] HDX-MS provides information about protein conformation and dynamic by measuring the rate of solvent where hydrogens are exchanged with the heavy hydrogen isotope deuterium.[57] XL-MS is a method used to elucidate the interactions in complex molecules by binding the residues in close proximity via covalent bound. XL-MS method will be explained in detail in following sections.

1.4 Cross-linking Mass Spectrometry (XL-MS) for Proteomics

Cross-linking mass spectrometry (XL-MS) is an evolving method for structural proteomics analyses, with constant improvements in the reagents, software, and instrumentation.[58] The method is used for the structural elucidation of protein complexes and determination of protein-protein interactions. XL-MS enables us to capture proteins from their native environment and provides information about physical contacts of the proteins by the use of agents (cross-linkers) binding proteins in close proximity. It allows obtaining different protein forms and it is capable of

providing data on proximal distances that could not be easily achieved by other structure determination methods.

The overall workflow, which is demonstrated in **Figure 1.5**, involves the selection of system to work on, cross-linking reaction, sample processing, chromatographic separation, MS analysis, cross-link identification, and analysis of cross-link data.[59] The proteins of interest are produced with appropriate methods, as described in **Section 1.3.1**, and then cross-linked with cross-linkers via covalent bonding. The cross-linked proteins are enzymatically digested and subjected to analysis using a high-resolution mass spectrometer. The resulting raw mass spectrometry data, which includes both precursor ions (MS) and product ions (MS/MS), is processed using cross-linking software to detect the proteins involved and the specific points of interaction. The identification of proteins is based on unique peptides searched against spectral and protein libraries.

The cross-linking reactions and the agents used will be reviewed in detail in the following sections.

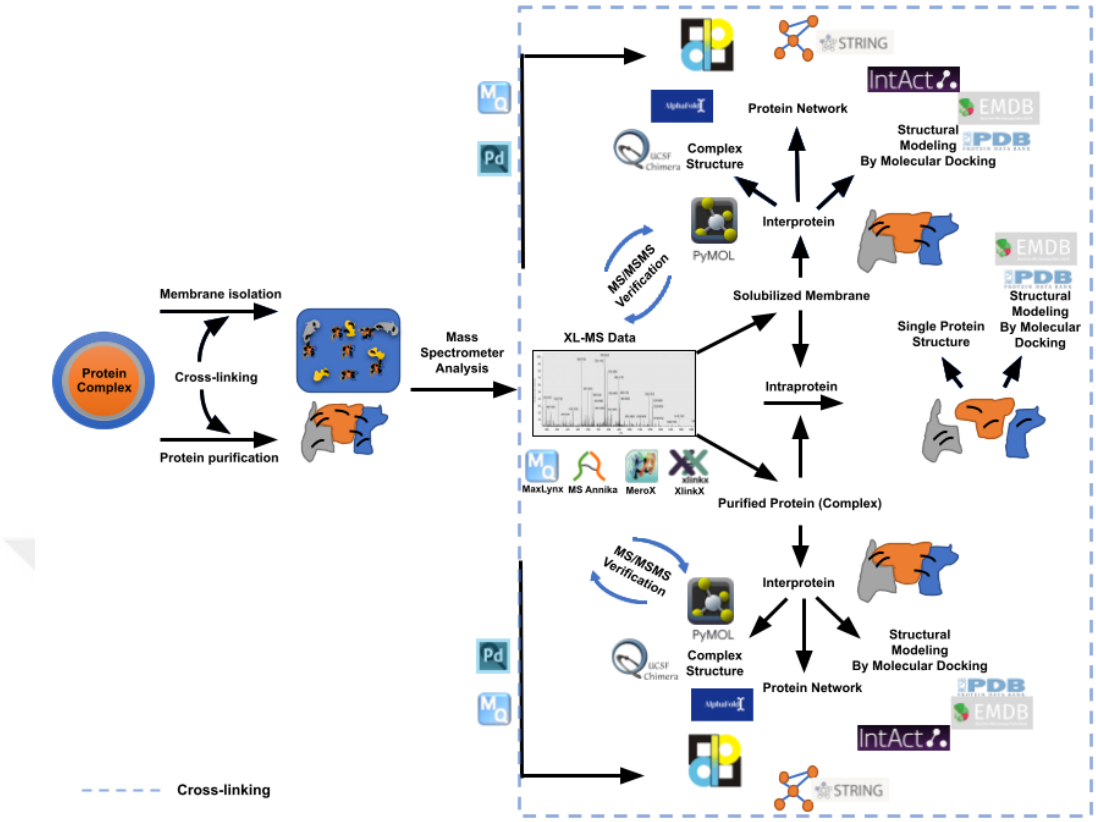


Figure 1.5. General workflow of cross-linking mass spectrometry (Akkulak et al., 2023).

1.4.1 Cross-Linking Chemicals and Process

Cross-linkers are chemicals that connect two specific residues of proteins with their reactive groups at the end of a spacer arm via covalent bond. Different cross-linkers exhibit different reactivity towards the side chains of amino acids. Amine-reactive cross-linkers, such as BS3 and DSBU, bind to lysine amino acid which has primary amines at the N-terminus and in the side chain. It is also suggested in the literature that NHS esters, such as DSBU, BS3, DSS, and DSSO, could show reactivity towards serine, threonine, and tyrosine amino acids.[60]

Cross-linkers are categorized based on their functions and MS features, as demonstrated in **Figure 1.6**. When their functions are considered, they could be homobifunctional or heterobifunctional. Homobifunctional cross-linkers possess

two identical reactive end groups and they are mostly used for intra-molecular cross-link formation with one-step reactions. Heterobifunctional cross-linkers, on the other hand, have different reactive groups at either end of spacer arm. The MS features of the cross-linkers change among the types. Some cross-linkers are cleaved when the fragmentation is applied, whereas others are not. The cleavable cross-linkers produce signature ions and the cross-linked peptide pairs are distinguished by their mass differences during the analysis. Non-cleavable cross-linkers, on the contrary, cannot be cleaved during fragmentation. The representative cleavable and non-cleavable cross-linkers are illustrated in **Figure 1.7**. The cleavable cross-linker DSBU and the non-cleavable cross-linker BS3 are two cross-linkers most commonly used in the literature.[58]

Besides the functionality and MS features of cross-linkers, targeted functional groups, spacer arm length, water solubility, and membrane permeability are the important factors of the selection process.

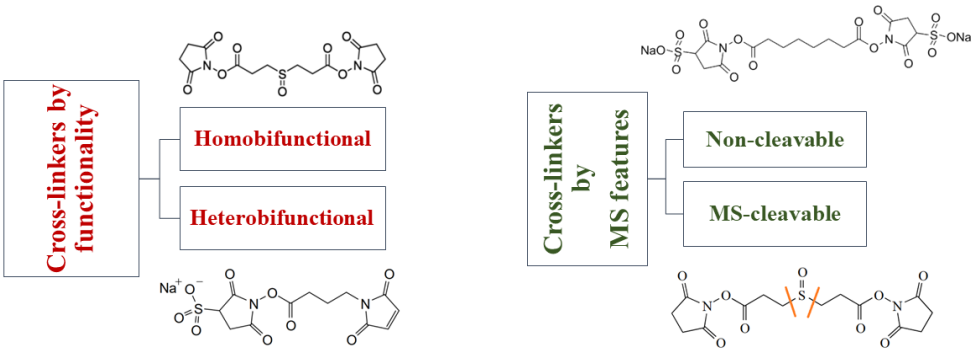


Figure 1.6. Categorization of cross-linkers by their functionality and MS features.

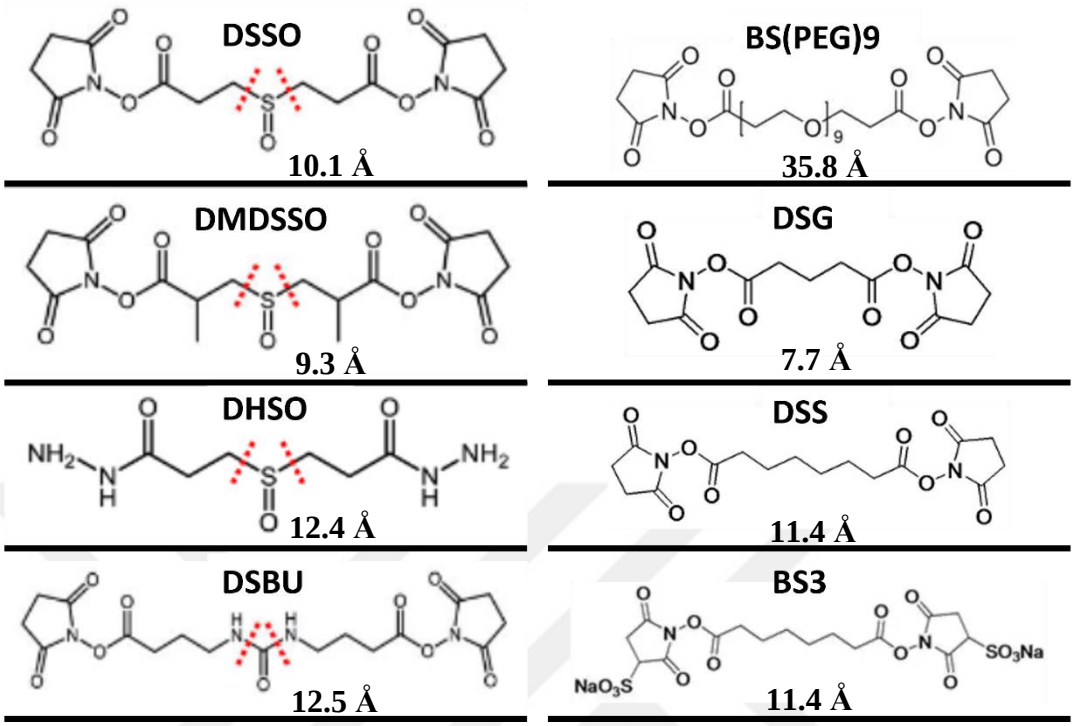


Figure 1.7. Representative MS-cleavable and non-cleavable cross-linkers, regenerated from "Cross-Linking Mass Spectrometry: An Emerging Technology for Interactomics and Structural Biology" by Yu et. al. Anal. Chem. 2018, 90 (1), 144–165.

Several types of connections are possible in the systems, as illustrated in **Figure 1.8**. The cross-linked products are distinguished as mono-links (type 0, dead-end), where one of the reactive end groups is bound to a residue, intra-molecular (type 1), where the cross-linker connects to different peptides in a protein, inter-protein (type 2), where the cross-linker connects to peptides of different proteins.[61] Cross-linkers are the key parts in the identification and characterization of cross-linked products. The generation of different types of products after the cross-linking reaction with appropriate cross-linkers leads to the accurate and reliable results.

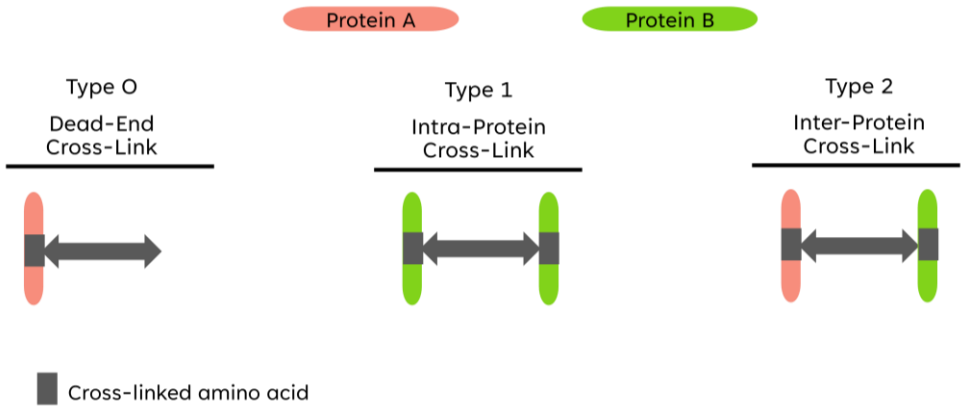


Figure 1.8. The three types of cross-linker connections.

1.5 State-of-the-art and Motivation

FtsH is known to form a mega complex with HflK and HflC. This complex plays a chaperone role to stabilize proteins in mitochondria and a modulator role to limit ATPase activity of FtsH. The structures of HflK and HflC are elucidated; however, the complete structure of FtsH protein in the complex is yet to be known.[17] Thus, this study has attempted to elucidate the structure of this complex by determining inter- and intra-protein interactions with XL-MS method, along with the attempt to decipher the protein-protein interactions of FtsH, HflK, and HflC. XL-MS is an integrative and complementary method to conventional structural biology methods used to obtain distance restraints and structural information[62]. It has been applied to purified proteins/protein complexes and a wide range of system-wide studies. Various cell lysates, including human cell lines[63–65] and Drosophila embryos[66], organelles[67,68], tissues[69,70], as well as living bacterial[52,71,72] and human cells[73–75], have been studied using this technique so far. In this study, the samples of solubilized membrane and purified protein of FtsH-HflK-HflC were cross-linked with DSBU and BS3, which are two commonly used cross-linkers for structural biology studies. The residues of proteins in close proximity were covalently bound to each other via cross-linkers. Cross-linked proteins were then enzymatically digested and analyzed by high-resolution mass spectrometer. The data obtained were then analyzed by four commonly used software, MaxLynx, MeroX, MS Annika, and XlinkX. XL-MS is an evolving method and there is no gold standard for software use. In the literature, the XL-MS software were compared using synthetic peptide libraries[76–79]; however, this study utilizes a real biological system for software comparison. Moreover, the software were compared by applying strict parameters (e.g. 1% of False Discovery Rate) in the studies so far[80]; however, in this study, the interactions were verified manually using raw MS and MS/MS data as well. The outcomes of this study will provide novel interactions of FtsH-HflK-HflC that could not be resolved and the physical interactions of FtsH, HflK, and HflC with other proteins to the literature. The roles of the FtsH-HflK-HflC complex in bacterial quality control mechanism

will be enlightened. Moreover, the overall workflow will guide researchers in identifying protein interactions by XL-MS and using XL-MS data.

CHAPTER 2

MATERIALS AND METHOD

2.1 Protein Expression and Purification

In order to obtain inter-protein and intra-protein interactions of the FtsH-HflK-HflC membrane protein complex two different samples were produced: Solubilized membrane and purified protein. Detailed information from transformation and over-expression to the purification is presented in the following sections.

2.1.1 Over-Expression of Proteins

- **FtsH-HflK-HflC Transformation and Over-expression**

A 50 µL of Lemo21 (DE3) competent *E. coli* cells were thawed on iced for 10 mins. A 1 µL of the plasmid (~100 ng total) (obtained from BvK LaB, TARLA) containing FtsH-HflK-HflC genes was added. Heat shock was given to the cells at 42°C for 10 seconds. The mixture was placed on ice for 5 mins. A 950 µL of LB broth was added and the mixture was placed at 37°C for an hour. A 20 µL of cells diluted 10-fold and 1 µL of ampicillin were spread onto the plate and incubated overnight. A single colony was picked for each and used to inoculate a 125 mL preculture with 100 µg/mL ampicillin and 50 µg/mL Spectinomycin in a 2L Erlenmeyer flask. The cells were grown overnight at 37°C while shaking. The 2xYT expression media was inoculated at 1:50 dilution. 20 mL of overnight culture was added to 1L of media in a 2L Erlenmeyer flask for over-expression. The cells were incubated at 37°C while shaking until 0.4-0.8 OD was reached. The cells were induced with 0.4 mM IPTG and 0.2% arabinose at 30°C for 4 hours. After 4 hours, the cells were harvested at 5000 rpm for 25 mins. The cell pellets were frozen till the separation and purification.

2.1.2 Cell Lysis

The same procedure was applied for all samples. The cell pellets were resuspended in 40 mL of the breaking buffer (50 mM HEPES, 100 mM NaCl, 2 mM of $MgCl_2$, 0.2 mg/mL lysozyme, 0.2 mg/mL RNAse, 0.2 mg/mL benzonase, 1 mM phenylmethylsulfonyl fluoride (PMSF), ½ tablet protease inhibitor) for 1L of culture. 12L of culture was used for the total protein used. The resuspension was incubated at 4°C for 30 mins using a magnetic stirrer. The cell pellets were sonicated with 40% amplitude, 2s on/8s off for 2 mins in 2 cycles. The sonicated cell pellets were centrifuged at 10.000 g for an hour, and the supernatant was taken.

2.1.3 Membrane Separation and Solubilization

The supernatant was centrifuged at 100.000 g for an hour to separate the membrane. The supernatant was discarded, and the waxy membrane pellet was weighed. 2% of n-Dodecyl-B-D-Maltoside (DDM) (w/w) and 15 mL of breaking buffer were added, and the samples were incubated at 4°C for an hour using a magnetic stirrer. Following the solubilization, the solubilized membrane was transferred to a prechilled ultracentrifuge tube and centrifuged at 100.000 g for 45 mins to separate the soluble part.

2.1.4 Protein Purification

3 mL Strep-tactin Sepharose (IBA Life Sciences) resin was used for 1L of culture and the resin was equilibrated using the wash buffer (100 mM HEPES pH 7.5, 150 mM NaCl, 0.01% DDM). The protein extract was added to the matrix slowly and incubation was done for at least 30 mins. The flow through was collected and the column was washed with 50 mL of wash buffer. The protein was eluted with 5 mL

of elution buffer (100 mM HEPES pH 7.5, 150 mM NaCl, 0.01% DDM, 2.5 mM desthiobiotin) 6 times. The elutions were concentrated separately to 200 µL using 100 kDa MWCO concentrators equilibrated with the wash buffer. The OD280nm and OD260/280 were measured with NanoDrop UV-Vis Spectrophometer (AllSheng).

2.2 Cross-Linking

Cross-linking reactions with disuccinimidyl dibutyric urea (DSBU) (Thermo Fisher Sigma, USA) and bis(sulfosuccinimidyl)suberate (BS3) (Thermo Fisher Sigma, USA) were carried out on the samples according to manufacturer's instructions.[50,63,81–83] A 50 mM of cross-linker stock solution was prepared with 46.9 µL of DMSO and 1 mg of DSBU. A 50 mM of cross-linker stock solution was, in addition, prepared with 70 µL of ddH_2O and 2 mg of BS3. 2.5 µL of cross-linker (final concentration of 10 mM) was added to 10 µL of solubilized membrane and 5 µL of cross-linker (final concentration of 2.5 mM) was added to 100 µL of purified protein sample. The mixture was incubated at room temperature for 60 mins. After the incubation. 20-fold Tris-HCl (1M, pH 8.0) was added to stop the reaction.

2.3 Proteolytic Digestion

The solubilized membrane and purified protein samples were extracted in 100 µL of 50 mM ammonium bicarbonate (pH 7.8) and incubated with 11 µL of 100 mM dithiothreitol (DTT) for 50 min at 60 °C to denature the proteins. Free cysteines were alkylated with 22 µL of 100 mM iodoacetamide (IAA) for 30 min at room temperature in the dark. Then 50 µL 0.1 µg/µL trypsin was added and samples were incubated at 37 °C for 18 h. Samples were dried in miVac at room temperature and dry peptide samples were dissolved in 0.1% formic acid. Before the MS analysis

peptide concentrations were determined with Pierce™ Quantitative Peptide Assay (Thermo Fisher Scientific, USA).

2.4 MS-based Proteomics Analysis

A 1 µg of digested samples (peptides) were analyzed by the UltiMate™ WPS-3000RS nanoLC system coupled with Orbitrap Fusion Lumos (Thermo Fisher Scientific, USA). The peptides were separated on Acclaim™ PepMap™ 100 C18 HPLC Columns (3 µm, 0.075 mm × 500 mm, Thermo Fisher Scientific, USA). The mobile phase A containing 0.1% aqueous formic acid and mobile phase B comprising 0.1% formic acid in 80% acetonitrile were set to a gradient; 0–5 min, 4–4% (B); 5–130 min, 4–35% (B); 130–150 min, 35–50% (B); 150–153 min, 50–100% (B); 153–168 min, 100–100% (B); 168–170 min, 100–4% (B); and 170–180 min, 4–4% (B). The MS and MS/MS spectra were collected with a mass range of m/z 300-1800 in positive ionization ion mode. The high-energy C-trap dissociation (HCD) fragmentation was performed with nitrogen gas, and collision energies of 25%, 30%, and 35%. The precursor and the product ions were detected at 120K resolution and 15K resolution, respectively.

2.5 Data Analysis

2.5.1 Protein Profiling

The raw MS data were analyzed via MaxQuant[41,42] through the whole *E. coli* proteome (ID: UP000000625) to profile the proteins present in the samples, using the parameters in **Table 2.1**.

- **MaxQuant Search Parameters**

MaxQuant parameters were optimized through several conditions applied for each run. The most protein group yielding, and the least decoy and contaminant existing parameters were applied during the analysis. The optimized parameters are shown in **Table 2.1**.

Table 2.1. Protein search parameters used for MaxQuant analysis.

Type	Parameter	Value
Group-specific Parameters	Variable Modifications	Oxidation (M), Acetyl (Protein N-term)
	Fixed Modifications	Carbamidomethyl (C)
	Enzyme	Trypsin
	Max. Missed Cleavages	1
	Max. Charge	4
	Main Search Peptide Tolerance	5 ppm
	First Search Peptide Tolerance	20 ppm
Global Parameters	Mass Spectrometry (MS/MS) Match Tolerance	20 ppm
	Min. Peptide Length	7
	Max. Peptide Mass	4600 Da
	Protein FDR	0.01
	PSM FDR	0.01
	Protein Quantification	Unique Peptides

- **Intensity Normalization**

Proteins obtained from MaxQuant analysis were compared based on their intensity. The intensities of each protein were divided by the sum of the intensities and then

the % ratio was calculated for relative protein quantitation. Proteins were sorted from high abundance to low abundance.

- **Gene Ontology (GO) Search**

The GO annotation search was carried out on STRING (v.11.5)[84] by applying advanced settings of required score as highest score (0.900) and FDR Stringency as 1%. The EnrichmentMap, which is a Cytoscape (v.3.9.1)[85] App, was used for the visualization of the results of gene-set enrichment. Generic enrichment results were used for operation and p-values were operated for visualization as radial heat-maps. Gene sets are represented by nodes and the sizes show the number of genes.

2.5.2 Structural Proteomics

In the structural proteomics part of the data analysis, the most commonly used four software were utilized for the cross-linking search to identify inter- and intra-protein interactions. The raw MS data were pre-processed when it is required, as described below. The search by the software was completed through target protein sequences and whole proteome. The interactions obtained were further manually verified using the raw MS and MS/MS data. The processes can be found in detail in the following sections.

2.5.2.1 Data Pre-processing

- **Conversion of Raw MS File**

The data format required by the software to process the data and identify cross-linked peptides varies from software to software. The raw data were used for MaxLynx[79], MS Annika[78] and XlinkX[77] without any conversion, whereas the data were converted into mzml file for MeroX.[76,81,86,87] In order to convert raw data to mzml ProteoWizard[88] software library was used.

- **Target Protein Sequences - FASTA**

Processing through the software was carried out by using the FASTA file comprising the sequences of FtsH, HflK, HflC and by retrieving whole *E. coli* proteome. UniProt was used to retrieve FASTA files. First, FASTA files of these four proteins were downloaded from the UniProtKB database and proteome of *E. coli* was downloaded from Proteomes database.

2.5.2.2 XL-MS Search

- **Software Workflow Integration**

The four software implemented to this study for the cross-link search were MaxLynx, MeroX, MS Annika, and XlinkX. MaxLynx is integrated into the MaxQuant environment. MS Annika and XlinkX are the two nodes of Proteome Discoverer (Thermo Fisher Scientific, USA). They both have different workflows that must be integrated into Proteome Discoverer 2.5. MS Annika workflow is present at the website of MS Annika, whereas XlinkX workflow is directly present in Proteome Discoverer 2.5 Cross-linking workflows. MeroX is specified for cross-linked peptide identification; therefore, they have their own specific algorithm of the software.

- **Search Parameters**

The overall cross-linking search parameters are presented in **Table 2.2**. C-terminals of lysine and arginine residues were defined as specific protease cleavage sites, and maximum missed cleavages were set to 3. Precursor and fragment ion mass tolerances were limited to 10 ppm and 20 ppm. Carbamidomethylation at cysteine was assigned as the fixed modification, while the oxidation at methionine was defined as the variable modification. Signal to noise ratio was set to 2.0. The minimum precursor mass limit was 1000 Da, whereas the maximum precursor mass limit was 20000 Da. The default settings were used instead. The cross-link modification sites were set as Lysine, Serine, Threonine, and Tyrosine in MeroX,

MS Annika and XlinkX; however, only Lysine was able to be chosen in MaxLynx. False Discovery Rate (FDR) was defined as 1%. The maximum Cα-Cα distance was defined as 30Å as suggested by previous studies.[87,89,90]

Table 2.2. Cross-linking search parameters.

Minimum Precursor Mass Limit	1000 Da
Maximum Precursor Mass Limit	20000 Da
S/N Threshold	2
Precursor Mass Tolerance	10 ppm
Fragment Mass Tolerance	20 ppm
Maximum Missed Cleavage	3
Minimum Peptide Length (AAs)	5
Maximum Peptide Length (AAs)	40
FDR	1%
Maximum Cα-Cα Distance	30Å
* Other parameters were kept as default parameters for all the software.	
** The corresponding parameters for all the software are applied as above.	

- **Computer Specifications**

The specifications of the computer used were Intel i5 9300-H Processor with 4 Cores (8 Logical Cores), and 8GB DDR4 2666MHz RAM. The maximum RAM usage was limited to 5GB of RAM (3GB RAM was assigned for the operating system). The capacity of the processor and the amount of free RAM Memory amount were two limiting factors.

2.5.2.3 Data Verification

The spectra obtained from different software were verified in MS and MS/MS levels through the raw MS file. FreeStyle software (Thermo Fisher Scientific, USA) was used to visualize and qualitatively analyze raw mass spectrometry data. First, MS/MS spectra, where the peptide fingerprints and cross-linker diagnostic ions exist, given by software were matched with the raw data. Then, m/z value of two peptides and cross-linker (MS spectra) were examined by charge states.

2.5.3 Protein-Protein Interactions (PPIs) Search

In order to understand the physical and functional interactions of complex proteins in biological system, STRING (v.11.5)[84], IntAct[91,92], DIP[93], MINT[94] and Cytoscape (v.3.9.1)[85] were used. The search on STRING was done by applying advanced settings of required score as highest score (0.900) and FDR Stringency as 1%.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Protein Expression and Identification

All the raw MS data were analyzed by MaxQuant to identify proteins in the purified protein and solubilized membrane samples prior to the cross-linking search. MaxQuant is designed for qualitative and quantitative analysis of protein isolates as well as complex mixtures. The algorithms of MaxQuant include the detection of precursor ions (MS), the verification of peptides (MS/MS) searching against the database and identification of proteins based on unique peptides.

The protein search parameters used for the MaxQuant analysis are given in **Table 2.1**. The protein search was carried out against *E. coli* proteome on purified protein and solubilized membrane samples cross-linked with DSBU and BS3. The number of proteins obtained from the DSBU cross-linked samples of purified protein and solubilized membrane was 160 and 511, respectively (**Appendix B**). The normalized % ratio of FtsH in purified protein sample was 70%, followed by HflK with 4% and HflC with 3%. However, the runs could not be completed in the samples cross-linked with BS3. Since the matrix used in this study was very wide and complex, the analysis through whole proteome of *E. coli* (4403 proteins) was difficult for the software to handle. This problem can be solved with the use of a super computer; however, it is not feasible for routine use. The complexity of the samples can be understood from the normalized TIC (Total Ion Chromatogram), which is presented in **Appendix C**. Since the mass of peptides is high when non-cleavable cross-linkers are bound, the ionization of the peptides and intensity of the peaks decrease[95], as can be shown in **Figure 3.1**. In addition, fragmentation of non-cleavable cross-linkers occurs randomly from carbon-carbon bonds, and the resulting carbocations are not stable. The intensities of these fragments are low and there are lots of random peaks.

Thus, the identification of non-cleavable cross-linkers is challenging for the software.

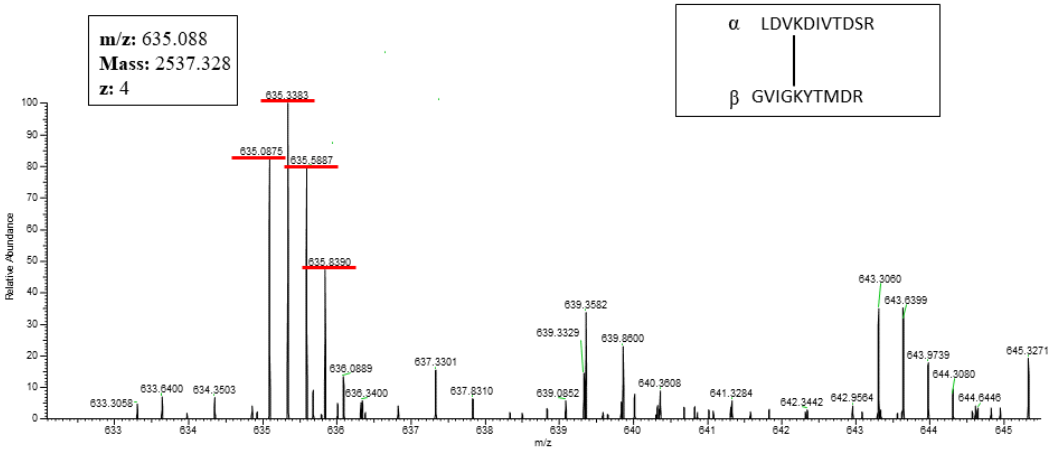


Figure 3.1. The representative MS spectrum of BS3-cross-linked two peptides (alpha and beta).

3.2 Cross-linker Assessment

In order to investigate the difference between two commonly used cross-linkers in the literature, MS cleavable cross-linker DSBU (disuccinimidyl dibutyric urea) and MS non-cleavable cross-linker BS3 (bis(sulfosuccinimidyl)suberate) (**Figure 1.7**) were chosen. The performance of these cross-linkers during the identification of inter-protein and intra-protein interactions was tested.

The samples were analyzed through the *E. coli* proteome by four cross-linking software, MaxLynx, MeroX, MS Annika, and XlinkX to elucidate the inter- and intra-protein interactions, and obtain the protein-protein interactions (PPIs). The analysis of the samples cross-linked with DSBU were completed by all software, yielding comprehensive and valuable interactions. The total number of inter-protein and intra-protein interactions of complex proteins and interactions with other

proteins was 444, as will be described in detail in **Section 3.3**. However, the analysis of the samples cross-linked with BS3 could not be completed in all software.

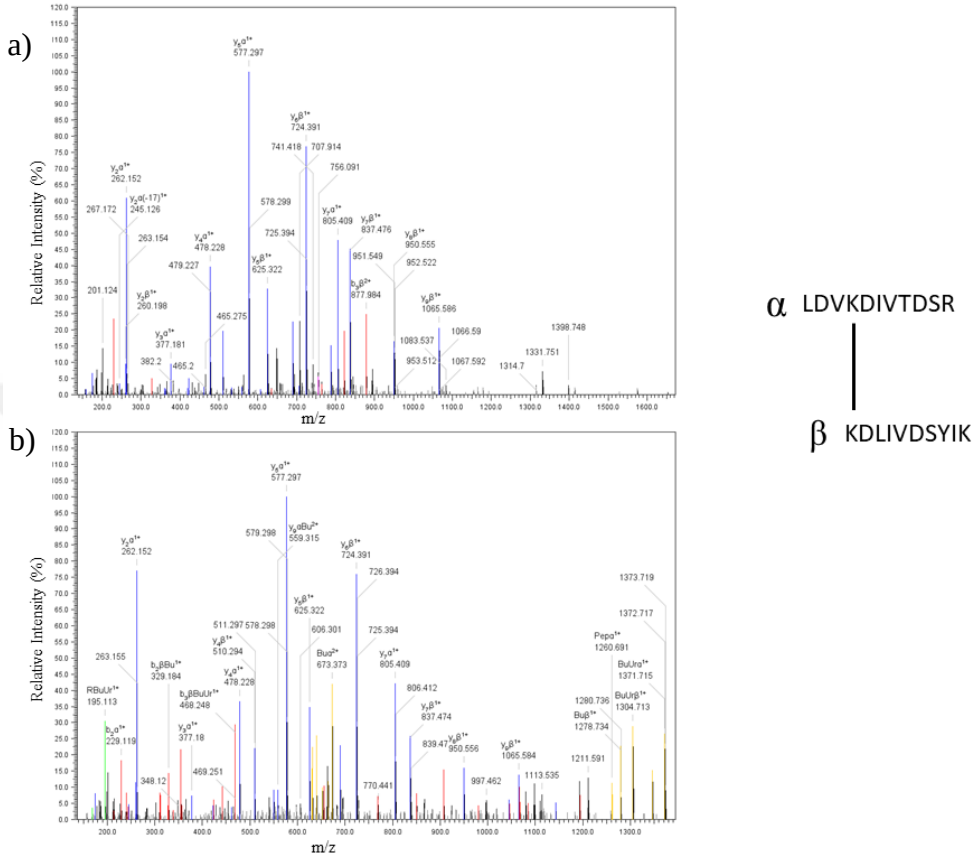


Figure 3.2. Representative MS/MS spectra of the purified protein sample of FtsH-HflK-HflC complex cross-linked with a) BS3 and b) DSBU.

The identification of peptide pairs cross-linked with MS cleavable cross-linkers in MS/MS is straightforward compared to the peptide pairs cross-linked with non-cleavable cross-linkers. The representative MS/MS spectra of the purified protein sample of FtsH-HflK-HflC complex are demonstrated in **Figure 3.2**. Since MS cleavable cross-linkers yield two pairs of signature ions when fragmented, the search algorithms of the software can distinguish the ions of individual cross-linked peptides by the mass differences. Therefore, identification is improved with cleavable cross-linkers.[96] The fragmentation of peptide backbone is also enhanced with the MS-cleavable cross-linker.[97] The DSBU cross-linker fragments (Bu and

BuUr) and other fragmentation products representing two peptides are shown in **Figure 3.2b**. The fragmentation of peptides cross-linked with BS3 was random and it did not occur as much as the peptides cross-linked with DSBU in 3 FASTA search, as can be seen in **Figure 3.2a**.

The application of BS3 was not successful in the proteome-wide cross-link search since the runs could not be completed. The identification of peptide pairs with non-cleavable cross-linkers is very challenging especially when larger, and more complex samples are analyzed.[80] The bacterial membrane used in this study was very complex and the protein complex studied was considerably large. Through the identification of cross-linked peptide pairs, the search space for non-cleavable cross-linkers is in n-square time complexity. The number of peptide pairs that meet the precursor mass limit is quadratic over the linear peptide number in the database since the pairwise interactions are considered.[98] The quadratic time complexity increases with the proteome-wide studies. However, in the case of cleavable cross-linkers, the signature ions produced by cross-linked peptide pairs reduce the search space.[99]

The proteome-wide analysis with DSBU-cross-linked samples provided very comprehensive and valuable information about the protein-protein interactions. Considering the results described above, only the DSBU cross-linker was chosen to proceed with.

3.3 Protein Interactions in the Complex

3.3.1 Cross-link Identification and Verification

The intra- and inter-protein interactions within and between the large multimeric membrane protein complex were analyzed in the purified form and over-expressed in the solubilized membrane. In order to identify the inter- and intra-protein

interactions, signature fragment ions representing cross-linkers and peptides were searched through four software.

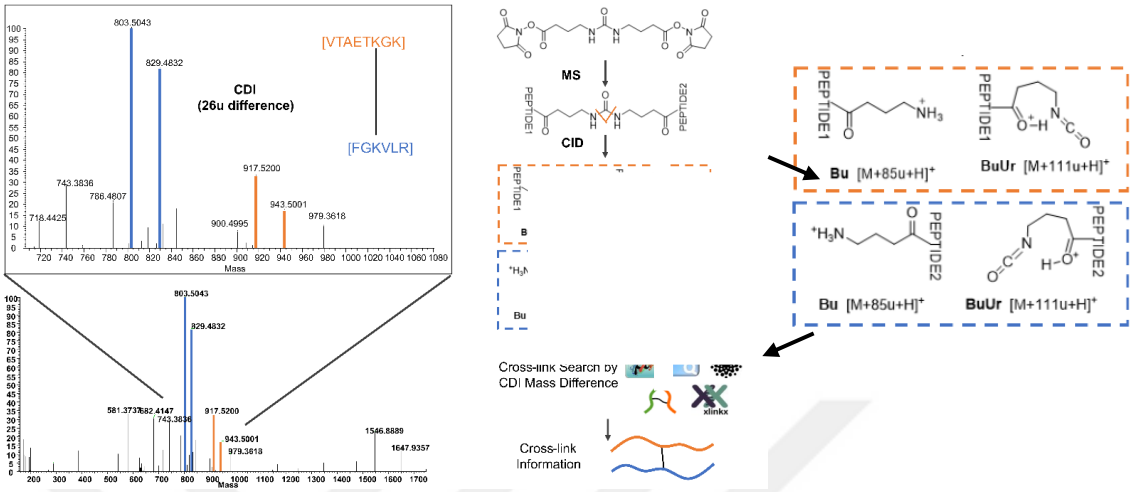


Figure 3.3. Fingerprint fragments confirming cross-linked peptides.

A representative MS/MS spectrum of a cross-linked peptide pair is presented in **Figure 3.3**. DSBU provided a significant advantage since software were able to confirm specific interactions using the mass difference between two fragments of cross-links, as mentioned before. The DSBU was covalently connected to residues of proteins using NHS ester reactive sides. The Collision-Induced Dissociation (CID) cleaves DSBU between carbonyl and amine parts and results in two characteristic diagnostic ions. The diagnostic ion corresponds to the peptide and cross-linker with protonated amine group at the cleavage point (PEPTIDE 1/2+Bu), which provides 85u mass adduct on the peptide. In **Figure 3.3**, the FGKVLR peptide with Bu part of the cross-linker on the lysine gives the peak of 803.5043. Additionally, the VTAETKGK peptide with Bu part of the cross-linker on the lysine peak appears at 917.5200. The other diagnostic ion is the BuUr part of the cross-linker bound to the peptide, where the nitrogen on amine group loses one proton due to the cross-linker cleavage, resulting in a 111u mass adduct to the connecting peptide (PEPTIDE 1/2+BuUr). **Figure 3.3** shows the FGKVLR peptide with BuUr

part of the cross-linker on the lysine giving the peak of 829.4832, and the VTAETKGK peptide with BuUr part of the cross-linker on the lysine peak appearing at 943.5001. The cross-link search was conducted by software to search 26 u mass difference owing to the cleavage of the cross-linker described above.

The XL-MS software predominantly uses lysine specific cross-linking sites to identify interactions. Literature also suggests that serine, threonine, and tyrosine could be cross-linked to DSBU.[60] However, corresponding connections have not been validated in tandem MS level up to date. Among the four software, only MeroX enables simultaneous multi/variable amino acid-crosslinker search. A representative fragmentation pattern confirming threonine-DSBU interactions is shown in **Figure 3.4**.

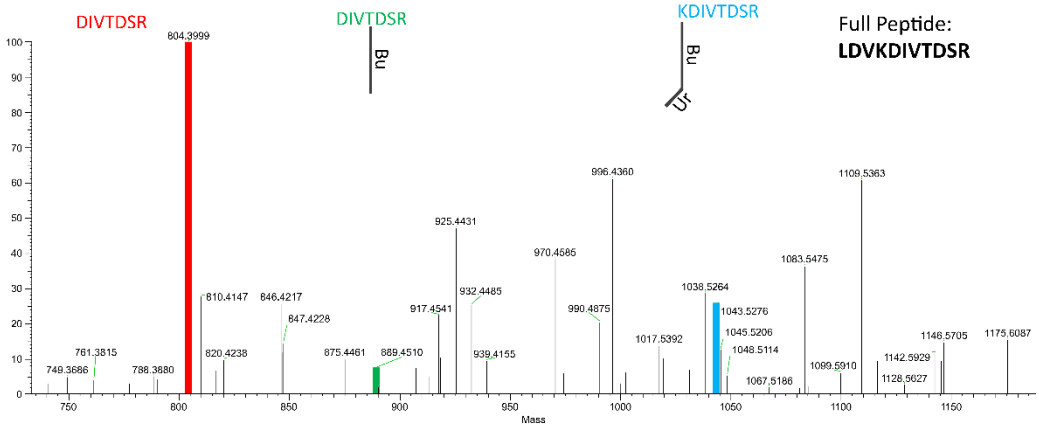


Figure 3.4. Different cleavage patterns of the cross-linker and backbone revealing the cross-link between threonine and lysine.

The deconvoluted MS/MS spectrum of LDVKDIVTDSR-GVIGKYTmDR peptide pair was given in **Figure 3.4**. In addition to two diagnostic ions specific to peptide-DSBU (85 and 111), several ions were observed to validate the threonine-

DSBU connection. The peak at the 1043.5276 mass unit belongs to Y8 cleavage of the peptide LDVKDIVTDSR and BuUr part of the cross-linker which is KDIVTDSR+BuUr. In this case, the cross-linker is bound to lysine. The peak at 889.4510 mass unit belongs to Y7 cleavage of the peptide LDVKDIVTDSR and Bu part of the cross-linker which is DIVTDSR+Bu. In this example, there is no lysine in the sequence, but threonine. Furthermore, the peak at 804.3999 mass unit belongs to Y7 cleavage of the LDVKDIVTDSR yielding the DIVTDSR without the cross-linker. Data suggested that the DSBU was linked to both lysine and threonine residues and corresponding peptides were co-eluted.[46]

The cross-link search through the whole proteome yielded 1093 unique interactions (**Appendix D**). The total number of inter-protein and intra-protein connections were 620 and 473, respectively (**Appendix K and L**).

3.3.2 Inter-protein Interactions

There were 30 inter-protein interactions obtained from four software and two different samples. Excluding repeating and unspecified connections from different software, 18 interactions between the complex proteins were found (**Table 3.1**). Two connections in the table shown as unspecific connections (198-141/143 and 294-328/330/331/333) confirm also that the unspecific connections are the link not on amino acid level, but in consecutive peptide levels allowing uncertainty up to 4 AAs. Four of the interactions were found through all four software considering both samples. Among them, one key interaction between FtsH and HflK was only found by MaxLynx from the purified protein sample. Considering that a single cross-link found can improve the quality of further studies such as modelling, this interaction is a valuable result[100]. The number of interactions between FtsH and HflC was 7, and the number of interactions between HflK and HflC was 12 from all the software and samples. The highest number of unique connections was obtained from the purified protein complex sample and most of them were by MaxLynx (11 interactions).

MeroX resulted in 5 unspecific connections from the purified protein complex and 2 from the solubilized membrane.

Table 3.1. Inter-protein interactions of three proteins in the complex obtained in two samples (PP: purified protein, SM: solubilized membrane) analyzed through four software, MaxLynx, MeroX, MS Annika, XlinkX.

		MaxLynx		MeroX		MS Annika		XlinkX	
	Connection Points	**PP**	**SM**	**PP**	**SM**	**PP**	**SM**	**PP**	**SM**
FtsH-HflK	31-133	+							
FtsH-HflC	84-120	+				+		+	
	61-211	+							
	60-211	+				+			
	31-63	+		+*		+		+	
	31-36					+			
	60-120					+			
	61 - 120		+						
HflK-HflC	172-36	+				+			
	346-327	+	+		+	+		+	
	172-122	+				+			
	294-327	+							
	198-137	+	+	+*	+	+	+	+	+
	294 -281	+						+	+
	198 - 141/143			+*	+*				
	294 - 248		+	+*	+*	+		+	+
	294 - 328/330/331/333			+*					
	198 - 82							+	
	198 - 84							+	
	310 - 281						+		+

*Unspecific connections

3.3.3 Intra-protein Interactions

A total of 119 intra-protein interactions were obtained from four software and two different samples, and 86 of them were non-repeating and unique interactions (**Appendix E**). There were 70, 14, and 2 intra-protein interactions of FtsH, HflC, and HflK, respectively from all the software and samples. In the purified protein sample, XlinkX gave the highest result with 39 interactions. The lowest number of unique intra-protein interactions was obtained by MS Annika in the solubilized membrane sample with 7 interactions. Fifteen of the interactions were found in all the software considering both samples.

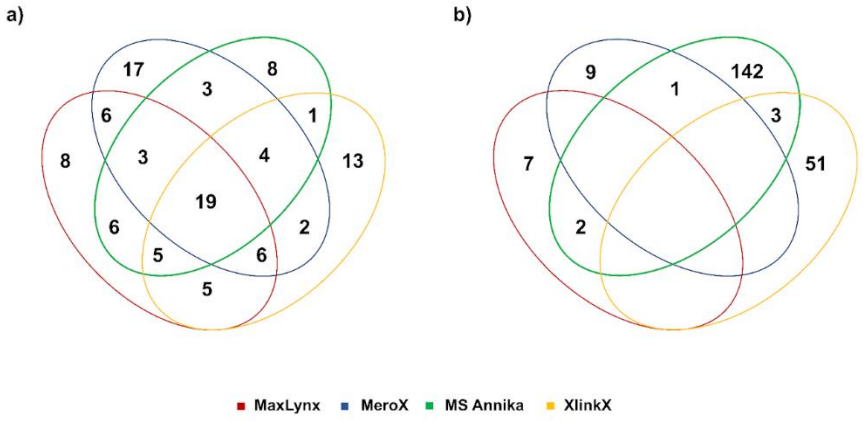


Figure 3.5. Venn diagram of interactions a) within and between complex proteins and b) interactions of complex proteins with other proteins.

The non-repeating and specific inter- and intra-protein interactions found in both samples were compared through all software based on the interactions within and between the complex proteins and the interactions of proteins in the complex with other proteins (**Figure 3.5**). Nineteen of the connections within and between the complex proteins were obtained in all four software. MeroX gave the highest result with a total of 60 connections, whereas MS Annika gave the least with 49

connections. MeroX, in addition, gave the highest number of interactions (17 interactions) not found in other software (**Figure 3.5a**). On the other hand, most of the interactions between the complex proteins and other proteins were obtained through MS Annika (**Figure 3.5b**). Compared with the previous results, MS Annika surprisingly caught a successful number of protein-protein interactions with 148 interactions including both samples. On the contrary, MaxLynx and MeroX gave the least number of interactions (9 and 10, respectively). Furthermore, there are not any common interactions found by all software. MeroX and MS Annika found 1 common interaction, MaxLynx and MS Annika found 2, MS Annika and XlinkX found 3. The utilization of the four software provided us deep insight about the protein-protein interactions in the samples. All of the interactions of complex proteins with other proteins can be found in **Appendix M**.

The FtsH-HflK-HflC complex was analyzed with the FASTA files of only these three proteins in all software for close-up examination of the interactions and for the comparison with proteome-wide search. The most abundant 10 inter- and intra-protein interactions from both samples were listed in **Appendix F**. Additional miscleavages and modified peptides were indicated in the table with different symbols. Since serine, threonine and tyrosine modifications were set as modification sides, it was clearly observed binding of the cross-linker to a residue other than lysine (**Figure 3.4**).

The interactions were further manually verified using raw data (MS and MS/MS) and 120 out of 160 interactions were successfully validated. The verification of results obtained from MeroX, MS Annika, and XlinkX was possible, whereas in MaxLynx the verification was difficult since the MS/MS spectrum could not be visualized in the software.

3.3.4 Visualization of Interactions on Protein Structures

Intra-protein and inter-protein interactions determined by various XL-MS software were visualized in previously published experimental structures and AlphaFold2-predicted models of the components of the membrane protein complex, FtsH-HflK-HflC in **Figure 3.6**. The cross-links between HflK and HflC, and their interactions between FtsH were confirmed in the recently published cryo-EM structure of the FtsH-HflK-HflC complex.[17] In the cryo-EM structure (PDB: 7WI3), HflK and HflC form a heterodimer with a close dimer interface, and the HflK-HflC dimer forms a dodecamer, comprising 12 HflK and HflC monomers. Thus, the cross-links between HflK and HflC may represent the connections between either the HflK-HflC heterodimer or neighboring HflK and HflC monomers. Moreover, cross-links between FtsH and HflK-HflC were determined only between the periplasmic region of FtsH, and stomatin/prohibitin/flotillin/HflK/C (SPFH) domain and N-terminal of HflK-HflC proteins, aligning with interactions observed in the cryo-EM structure. Both intra- and inter-protein cross-links obtained by MaxLynx and MeroX were well distributed on the experimental structure of the membrane protein complex; however, less number of interactions were determined between FtsH and HflK-HflC with MaxLynx and MeroX, compared to those obtained by MS Annika and XlinkX.

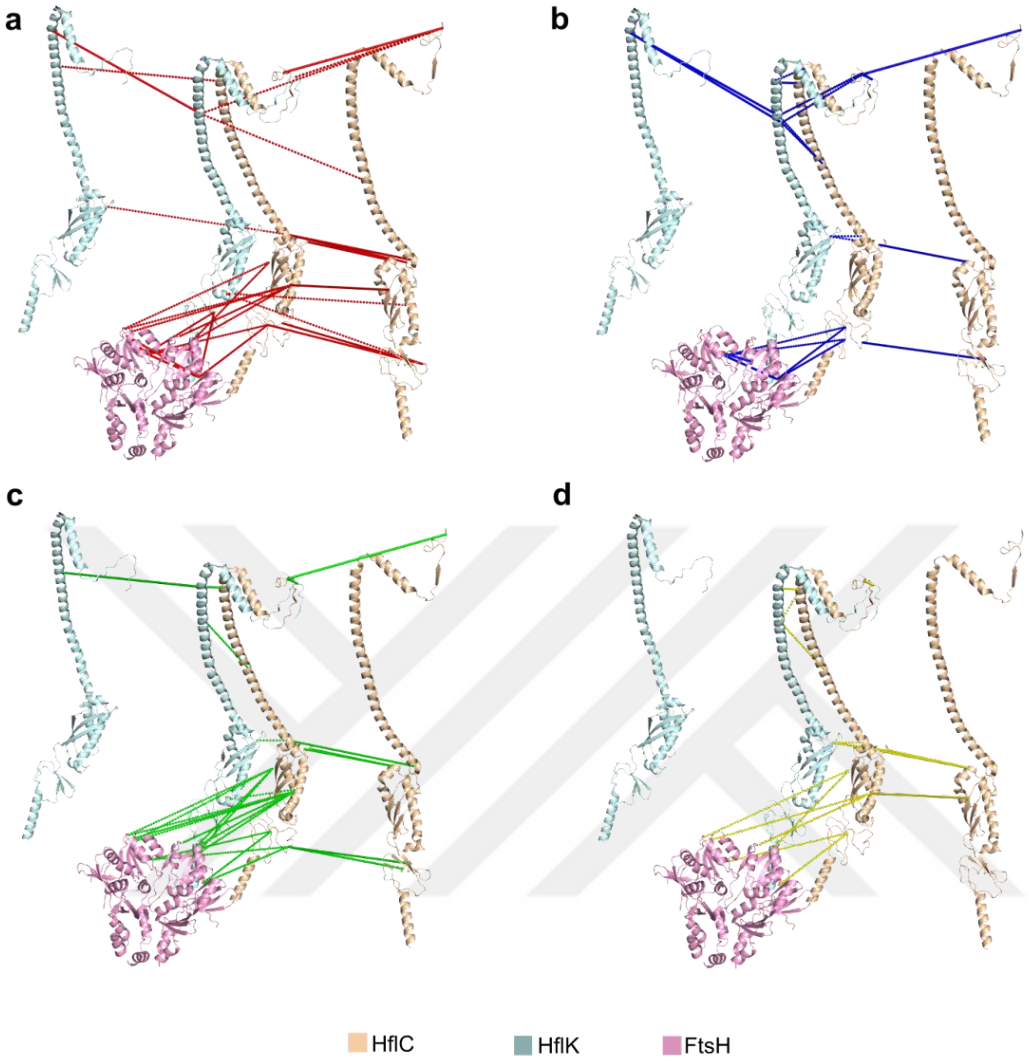


Figure 3.6. Top ten intra-protein (lines 1. Top ten intra-protein (lines) and inter-protein (dashed lines) interactions of FtsH-HflK-HflC (pink - light blue - wheat) membrane protein complex (PDB ID: 7WI3) obtained from XL-MS software and colored red MaxLynx (a), blue MeroX (b), green MS Annika (c) and yellow XlinkX (d). Periplasmic and transmembrane regions of hexameric FtsH are shown only. The possible interactions are also shown between neighboring HflK and HflC that are positioned away from the HflK-HflC heterodimer.

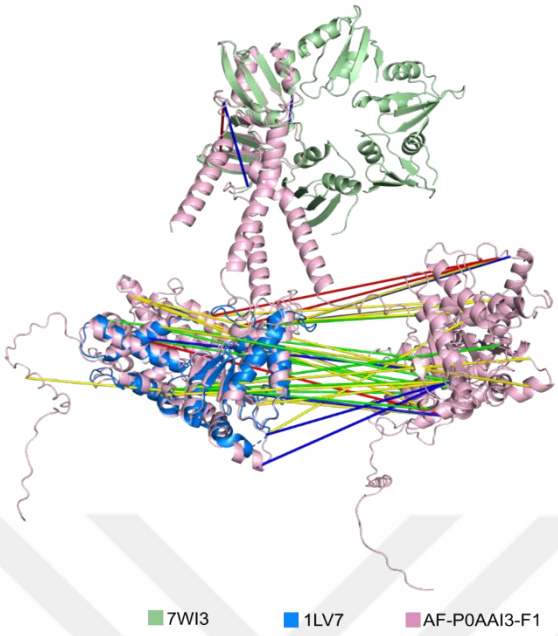


Figure 3.7. The detailed intra-protein connections of FtsH, represented between two monomers, obtained from XL-MS software MaxLynx (red), MeroX (blue), MS Annika (green) and XlinkX (yellow). Experimental structures are superposed with the predicted AlphaFold2 model (pink) (AF-P0AAI3-F1). The periplasmic region of hexameric FtsH (PDB ID: 7WI3) is shown in pale green, the partial cytoplasmic structure (PDB ID: 1LV7) is shown in blue. 91 residues of FtsH, in which no linkage was obtained, in N-terminus were removed in the AlphaFold2 model for clarity.

The intra-protein interactions in FtsH were visualized on both experimental structures (PDB: 1LV7, 7WI3)[17,101] and the predicted AlphaFold2 model (AF-P0AAI3-F1) due to the lack of full-length FtsH structure deposited in the PDB although experimental structures align well with the predicted model (**Figure 3.7**). There are cross-links determined between not only the periplasmic region (N-domain) but also the cytoplasmic region of FtsH. These connections may reflect both intra-protein interactions within the FtsH monomer and between FtsH monomers at the dimer interface as FtsH exists in a hexameric conformation. MaxLynx and MeroX determined cross-links within the N-domain of the FtsH whereas the number

of interactions within the cytoplasmic region were low compared to other software. On the other hand, there were many connections determined by MS Annika and XlinkX within the cytoplasmic region; however, there was no cross-link determined in the N-domain with these software.

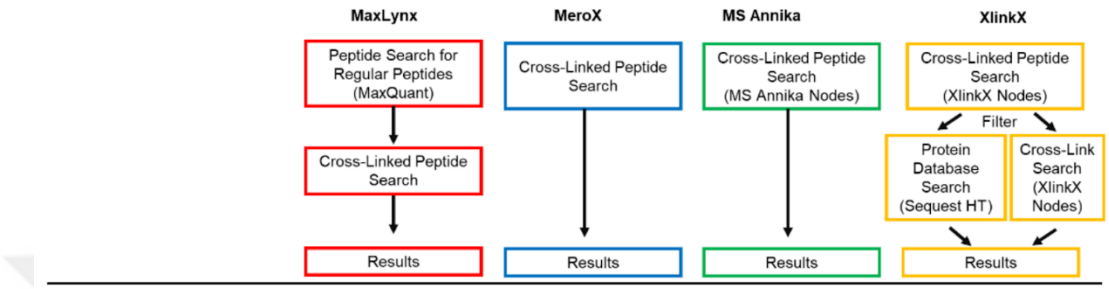
Overall, there are sufficient intra- and inter-protein interactions determined by each XL-MS software. The partial structure of FtsH was elucidated and interactions between residues which are not seen in experimental structures were found in this study. For instance, HflK-Lys360 interacts with FtsH-Lys408, HflC-Lys211 interacts with FtsH-Lys60, HflC-Thr330, Ser331, and Thr333 interact with HflK-Lys294 and Lys346. All these interactions may be used to model the protein complex using experimental and predicted structures, and molecular docking programs such as HADDOCK.[102] Multimeric structures of proteins, for instance, hexameric FtsH or dodecameric HflK-HflC, can also be modeled by molecular docking and cross-linking data. Likewise, the multimer structures predicted by structure prediction programs such as AlphaFold[103,104] and RoseTTAFold[105] can be confirmed by XL-MS data. The predicted structures can also be fitted into low resolution cryo-EM maps or SAXS (Small Angle X-ray Scattering) models.

In addition to structural proteomics studies, the interaction between proteins can be used for protein mapping and protein-protein networking, especially for complex samples such as a cell membrane as in our study.[106]

3.4 XL-MS Software Performance Characteristics

Four software were compared according to performance parameters; the number of cross-links identified, and processing time for two different datasets of solubilized membrane and purified protein complex (**Figure 3.8**). According to

overall time spent during data processing by the software, MS Annika was the fastest.



	MaxLynx	MeroX	MS Annika	XlinkX
Connecting Residues	K	K,S,T,Y	K(S,T,Y)	K
Processing Time (Mins)*	106 (SM) / 146 (PP)	32 (SM) / 23 (PP)	21 (SM) / 5 (PP)	62 (SM) / 96 (PP)
Complex Inter-Protein Interactions	13	11	11	10
Complex Intra-Protein Interaction	45	49	38	45
Complex Proteome-wide Interactions	9	11	148	54

Figure 3.8. Comparison of four XL-MS software; MaxLynx (Red), MeroX (Blue), MS Annika (Green), XlinkX (Yellow). Overall search process, including connection residues, processing time for solubilized membrane (SM) and purified protein (PP), complex inter-protein interactions, complex intra-protein interactions and complex proteome-wide interactions are given.

Proteins were analyzed based on unique peptides. The number of interactions is based on unique peptides connected via DSBU. The DSBU cross-linker was already integrated into all software. The software outcome file lists inter- and intra-protein interactions by indicating specific AA positions and corresponding tryptic peptide sequence for each protein pair. The matching score is also listed along with standard MS search parameters such as number of matching spectra, precursor, mass error, retention time and intensities.

MaxLynx is a software embedded into MaxQuant, a protein database search software, and thus, the cross-link search is performed only after the protein search. Although it is a useful process to identify proteins and their interactions simultaneously, the overall processing time is longer. This could be a limitation considering the size and spectra count of a high-resolution MS data searched against a large database.

The cross-linker connection side could be set to only lysine residue by default in MaxLynx, contrary to MeroX software which conducts only cross-linked peptide search at a time. Unlike MaxLynx and other software, MeroX enables selecting multiple residues such as serine, threonine, and tyrosine in addition to lysine simultaneously. Searching various cross-linking possibilities does not make MeroX's processing time longer, compared to MaxLynx and XlinkX. In addition, the graphical user interface (GUI) is user-friendly and fluent, and the size of data is not a limitation. However, in MeroX, the protein search is not an option, and only cross-linking information can be obtained.

MS Annika is a Proteome Discoverer 2.5 node, and the workflow used for MS Annika contains a spectrum selector mode. MS Annika allows conducting searches with multiple residues such as serine, threonine, and tyrosine in addition to lysine, but cannot achieve this simultaneously. Also, it contains both protein IDs and cross-link information. The processing time is significantly shorter than any other software. This might be due to the less number of nodes in the overall search workflow, and doing only cross-linked peptide search by MS Annika nodes. Additionally, MS Annika nodes provide different search modes and these modes result in fast processing times.

XlinkX is another Proteome Discoverer 2.5 node with a workflow consisting of different nodes, such as Sequest HT. Thus, protein database search is performed with the cross-link search. This is a useful outcome for identifying proteins in samples, and their interactions, like MaxLynx. This may cause the longer processing

time of XlinkX than MS Annika in a way. However, the cross-link search is performed with only lysine residue, and this is one of the limitations of XlinkX.

All the software provided similar numbers of inter-protein connections between complex proteins. MaxLynx provided 13, MeroX provided 11, MS Annika Provided 11, and XlinkX provided 10 inter-protein connections between complex proteins. Likewise, the number of intra-protein connections are similar. MaxLynx provided 45, MeroX provided 49, MS Annika Provided 38, and XlinkX provided 45 intra-protein connections within complex proteins. However, MS Annika yielded the highest number of cross-links (148 interactions) between complex proteins and other proteins, compared to all other software (**Figure 3.8**).

All the software have different source usage during data analysis (**Appendix G**). MaxLynx is the most source-friendly software in terms of CPU and RAM usage. While the RAM usage of MeroX was comparable to that of MaxLynx, its CPU usage was exceptionally high, reaching 99% utilization during analysis. On the other hand, MS Annika utilized all 5 GB of RAM assigned to it; however, had a CPU utilization of around 25%, lower than that of MeroX and XlinkX, and similar to that of MaxLynx. XlinkX also had a high CPU usage of 99% like MeroX, and utilized more RAM than MeroX, with an average of 3.8 GB, but still less than that of MS Annika. These differences can be due to the different search and working algorithms of each software. Additionally, it was observed that computer resources usage was the same for both purified protein and solubilized membrane MS data. However, the processing time changes depending on the file size.

3.5 Protein Network

3.5.1 Protein Search

The identification of proteins in the purified protein complex and solubilized membrane fractions allowed us to compare differences in interaction maps generated by cross-links. It was aimed to determine interactions not only within the FtsH-HflK-HflC membrane protein complex, but also with its surroundings. The network map generated by the STRING database revealed numerous connections, indicating that FtsH, HflK, and HflC have multiple biological relations with other proteins involved in different pathways. Upon comparing the two samples, two factors stood out: genomic neighborhood and functional similarity. The solubilized membrane sample contained 42% membrane protein, whereas the purified FtsH-HflK-HflC sample had an overall membrane protein content of 96% (**Appendix H**).

A total of 20 connections from the findings obtained with protein search of both samples were curated in the STRING, IntAct and DIP databases as functionally associated with FtsH, HflK, and HflC. Approximately half of the interacting partners were found in the purified sample (**Figure 3.9a**), while the solubilized membrane fraction had 17 interacting partners (**Figure 3.9b**). STRING, IntAct and DIP databases were used for the demonstration of functional association. RpIL, RpID, YajC, GpmA and RlpIU proteins were found to be associated only on DIP and IntAct, whereas all remaining interacting partners were found by all three databases.

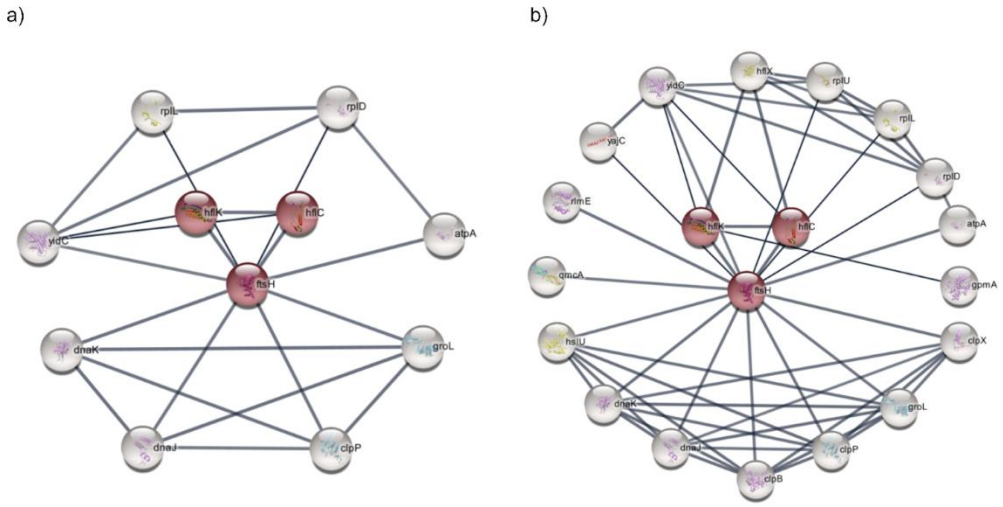


Figure 3.9. Interacting partners (colored in grey) of FtsH-HflK-HflC (colored in red) a) in the purified protein complex sample b) in the solubilized membrane fraction (STRING database combined with IntAct and DIP, score ≥ 0.9, FDR Stringency 1%).

Figure 3.10 and **Figure 3.11** show biological processes in which the proteins in the solubilized membrane and purified protein samples are involved, respectively. A total of 108 nodes in the solubilized membrane sample (**Figure 3.10**) and 60 nodes in the purified protein sample (**Figure 3.11**) were obtained. The top 15 biological processes with the lowest p-values are shown. Translation and peptide biosynthetic process are the biological processes with the lowest p-values. Proteins involved in translation, membrane protein insertion and quality control processes constitute 60% of all interacting partners identified in both samples (**Appendix I**). Several chaperones, including ClpB, DnaK, DnaJ, GroEL, and proteases such as ClpP, ClpX, HslU, as well as YidC, and YajC, were identified in both samples. These chaperones are functionally associated with the FtsH-HflK-HflC membrane protein complex**.** Specifically, YidC and YajC are proteins involved in preprotein translocation, and a

part of the membrane-bound holo-translocon system comprising seven proteins including SecB (chaperone), SecA (ATPase), SecYEG (protein secretion complex), and two membrane proteins, SecD and SecF, playing roles in the release of the mature peptide into the periplasm. The proximity of YidC to FtsH, HflK, and HflC in the bacterial cell was previously shown via *in vitro* photo cross-linking. The confirmed interaction of YidC with the FtsH-HflK-HflC complex, and coexistence of YajC with it suggest that FtsH-HflK-HflC complex interacts and cooperates with the holo-translocon system in membrane protein secretion and quality control.

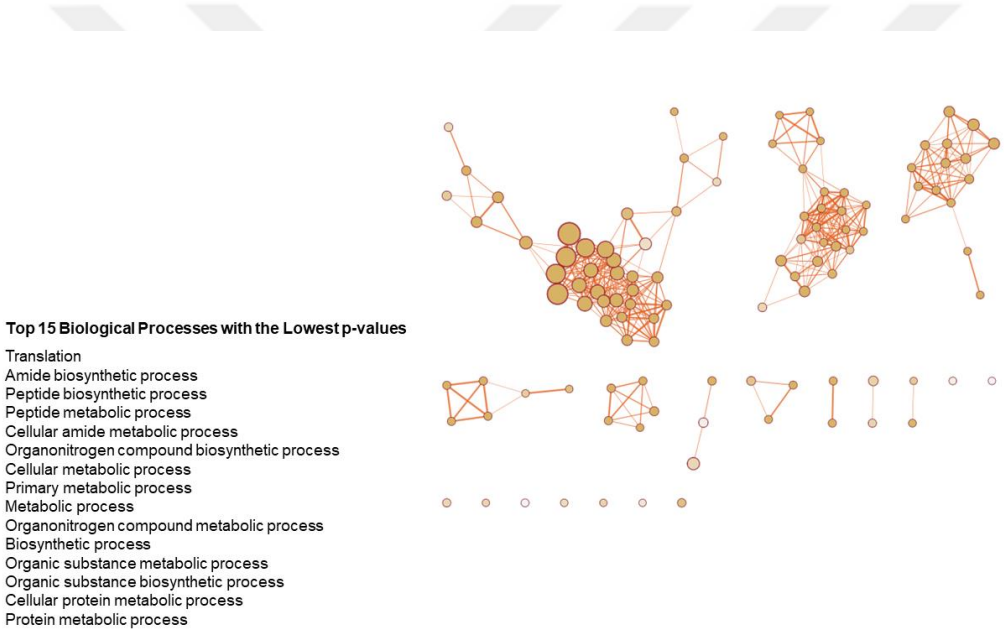


Figure 3.10. The top 15 biological processes of proteins found in the solubilized membrane sample with the lowest p-values.

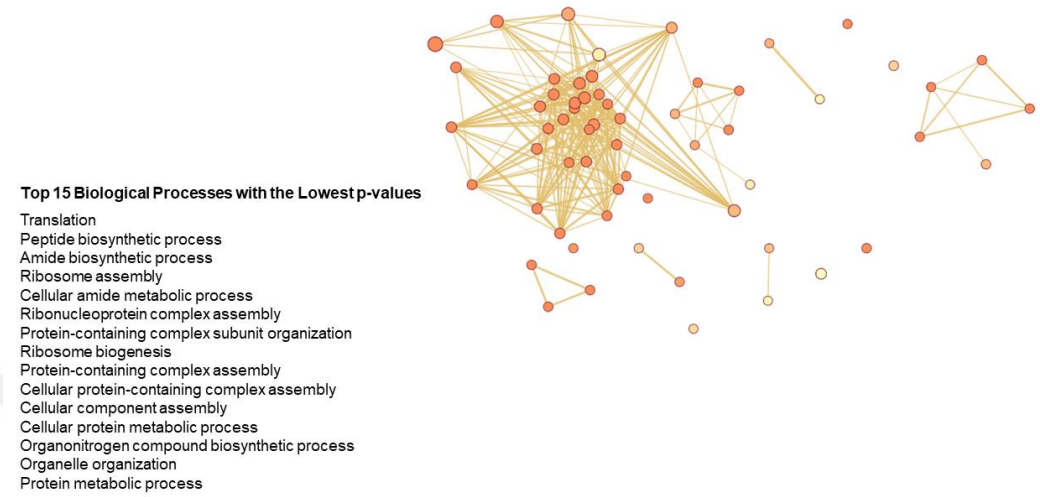


Figure 3.11. The top 15 biological processes of proteins found in the purified protein sample with the lowest p-values.

3.5.2 Cross-Linking

A total of 106 interactions of HflC, 96 interactions of FtsH, and 23 interactions of HflK, including FtsH-HflK-HflC complex, with other proteins separately from the cross-linking analysis of both samples were obtained through four software. Approximately 93% of the interactions were found in the purified complex sample and 223 interactions (out of 225) were verified through the raw data by fragment (MS/MS) and precursor ions (MS). These interactions were compared with literature through online databases, STRING (https://string-db.org), IntAct (https://www.ebi.ac.uk/intact/home), DIP (http://dip.doe-mbi.ucla.edu), and MINT (https://mint.bio.uniroma2.it/). The connections of FtsH, HflK, and HflC were presented (**Appendix J).**

Although the literature suggests that GroEL, ClpB, and HflX are functionally associated with HflC, direct interactions between these proteins and HflC have been

identified. Chaperonins such as ClpB and GroEL play an essential role in protein folding, particularly under stress conditions where unfolded proteins are generated. Therefore, the novel direct interactions of ClpB and GroEL with HflC may be responsible for the exposure to stress during the expression of the recombinant FtsH-HflK-HflC membrane protein complex. Another novel interaction of HflX to HflC indicates a possible cooperation between these proteins according to their regulating role for protein synthesis and quality control of membrane proteins.[86]

CHAPTER 4

CONCLUSION

In this study, the composition and structure of FtsH-HflK-HflC complex were investigated with the XL-MS based structural proteomics. Commonly used XL-MS software tools, MaxLynx, MeroX, MS Annika, and XlinkX were utilized to elucidate the protein interactions within a membrane protein complex containing FtsH, HflK, and HflC, over-expressed in *E. coli*. The four software were assessed based on the utility, feasibility and reliability and the yield of inter- and intra-protein connections. In addition, two common cross-linkers in literature were assessed. Given that the affinity-based purified complex yielded more interactions with other proteins, FtsH-HflK-HflC complex may be interacting with many associated proteins in the cell. The outcomes of the study were compared with the complex structure obtained from Protein Data Bank (PDB) to validate intra- and inter-protein interactions. Interactions between residues not seen in experimental structures were also determined. These interactions may be used to model monomeric and multimeric protein complexes using experimental and predicted structures, and molecular docking programs, assisted with protein structure/assembly prediction programs. Outcomes were not only the cross-link information within the overexpressed FtsH-HflK-HflC complex but also interactions between other proteins in close proximity with the protein and the protein complexes. Thus, XL-MS can be used for protein mapping and protein-protein networking studies in various matrices. Further studies are ongoing to elucidate the structural and biological phenomena behind the protein-protein interactions and decipher the possible complexes of FtsH.

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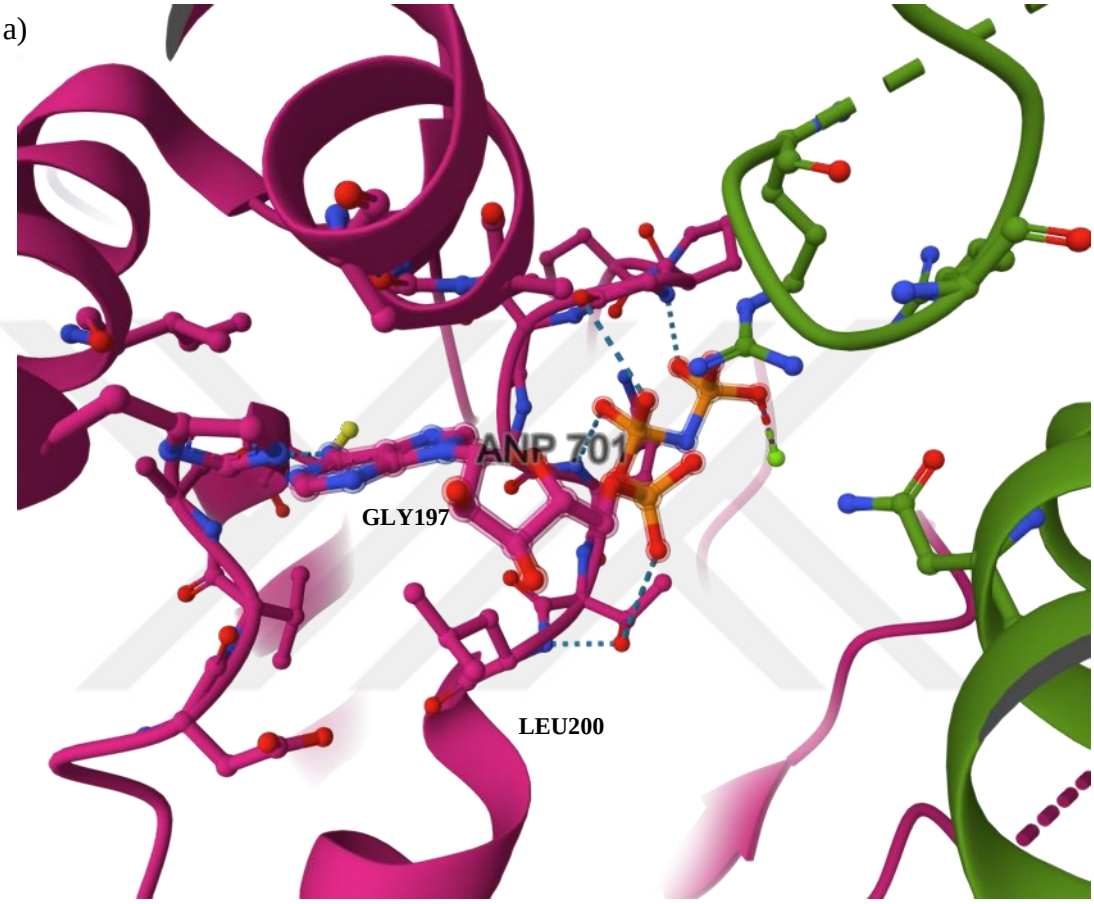
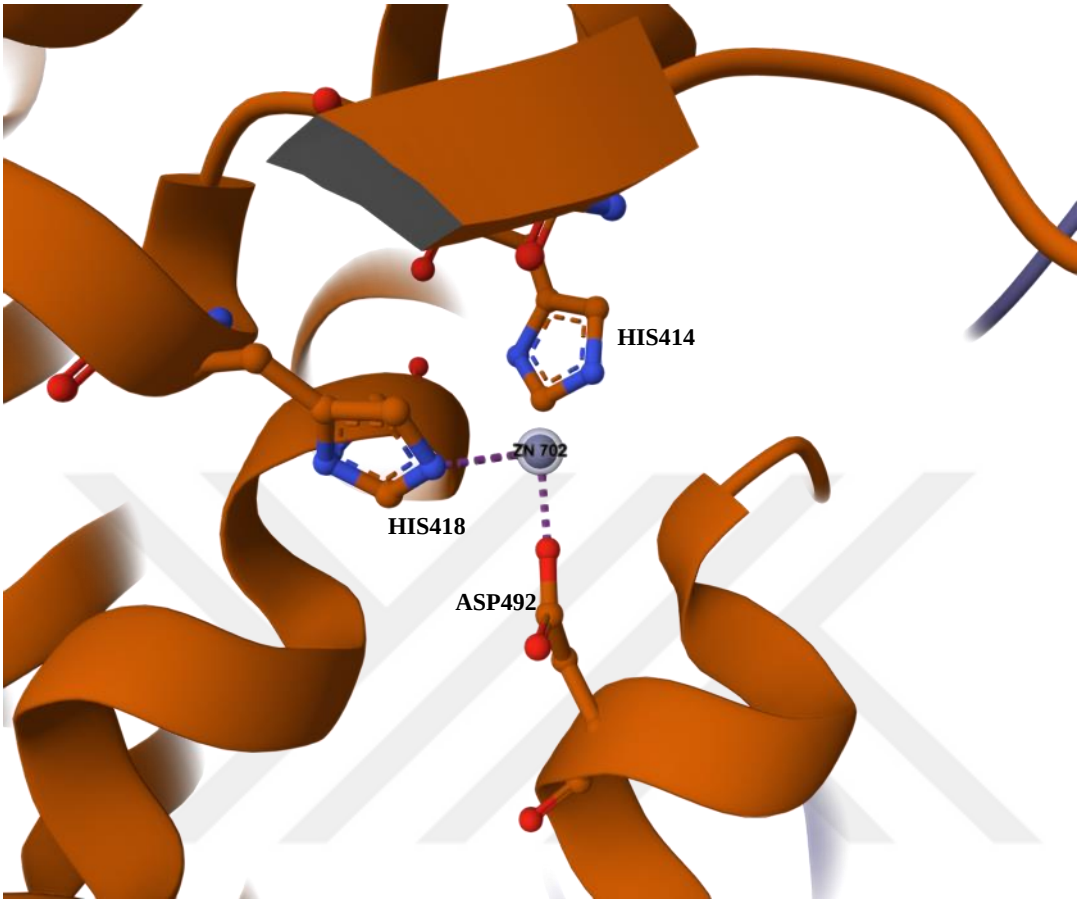
APPENDICES

A. a) ANP (phosphoaminophosphonic acid-adenylate ester) and b) Zinc-binding domains of FtsH.

a)

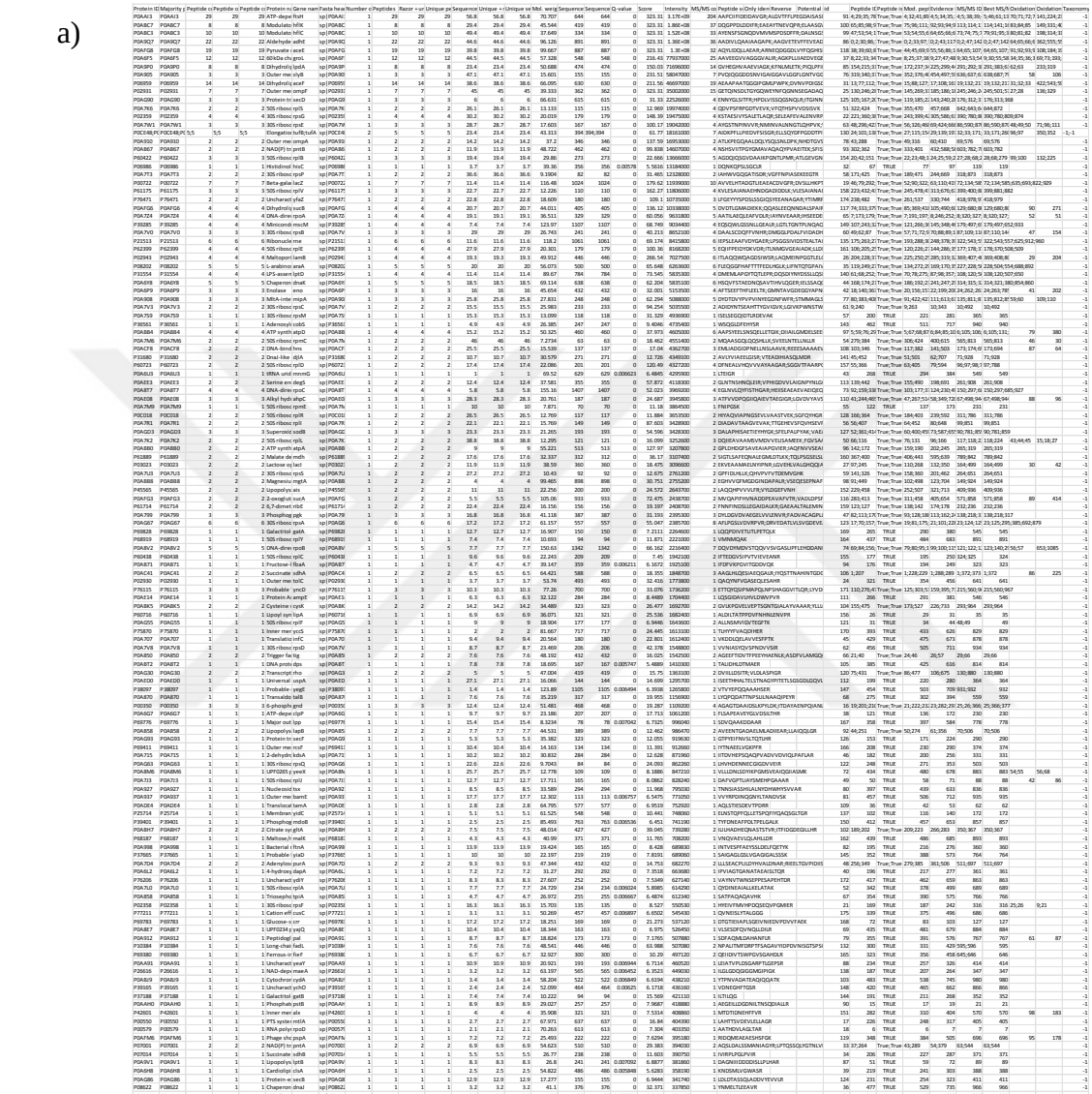


b)

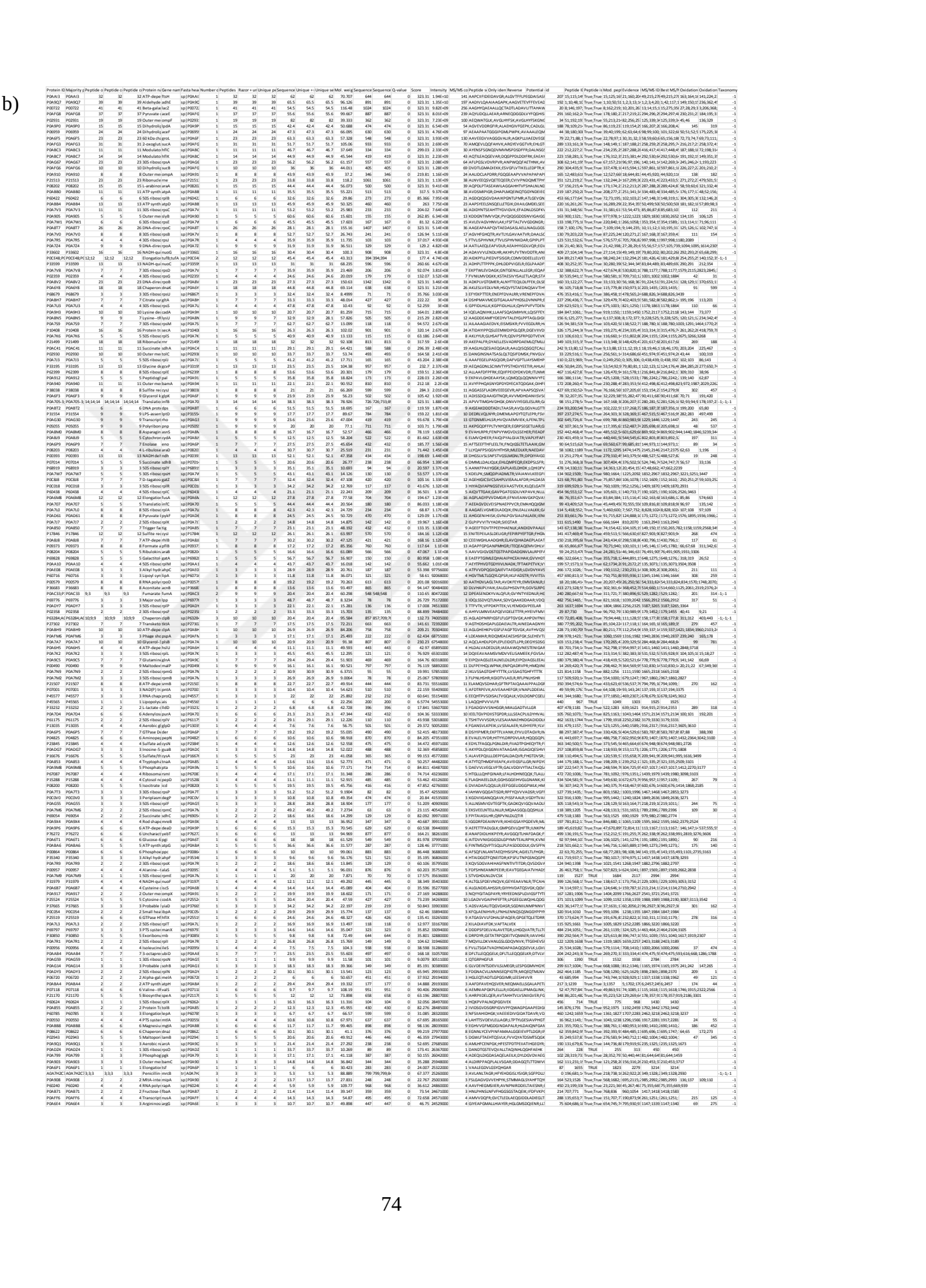


B. Protein search results of DSBU-cross-linked a) purified protein and b) solubilized membrane samples of FtsH-HflK-HflC complex.

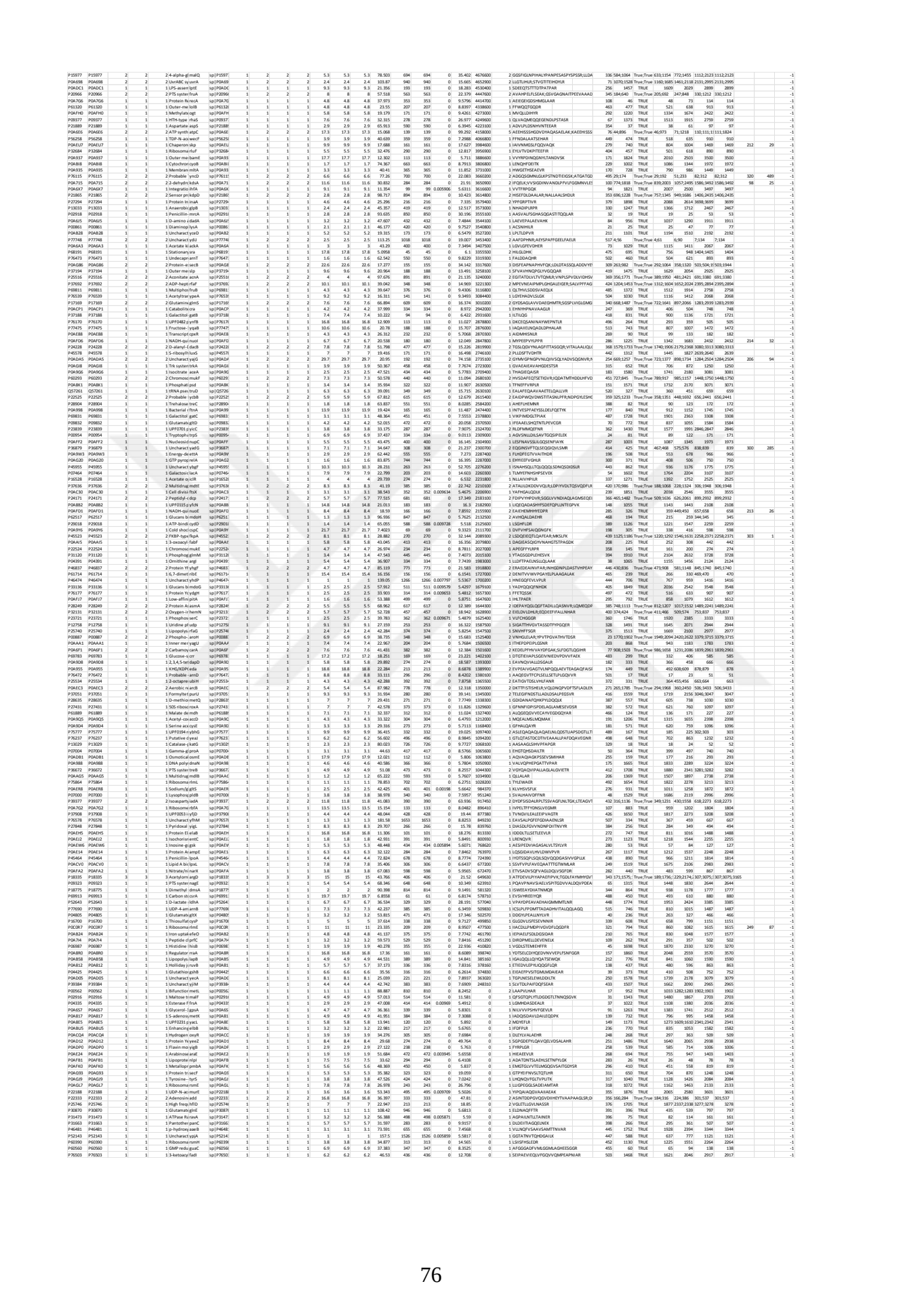
a)



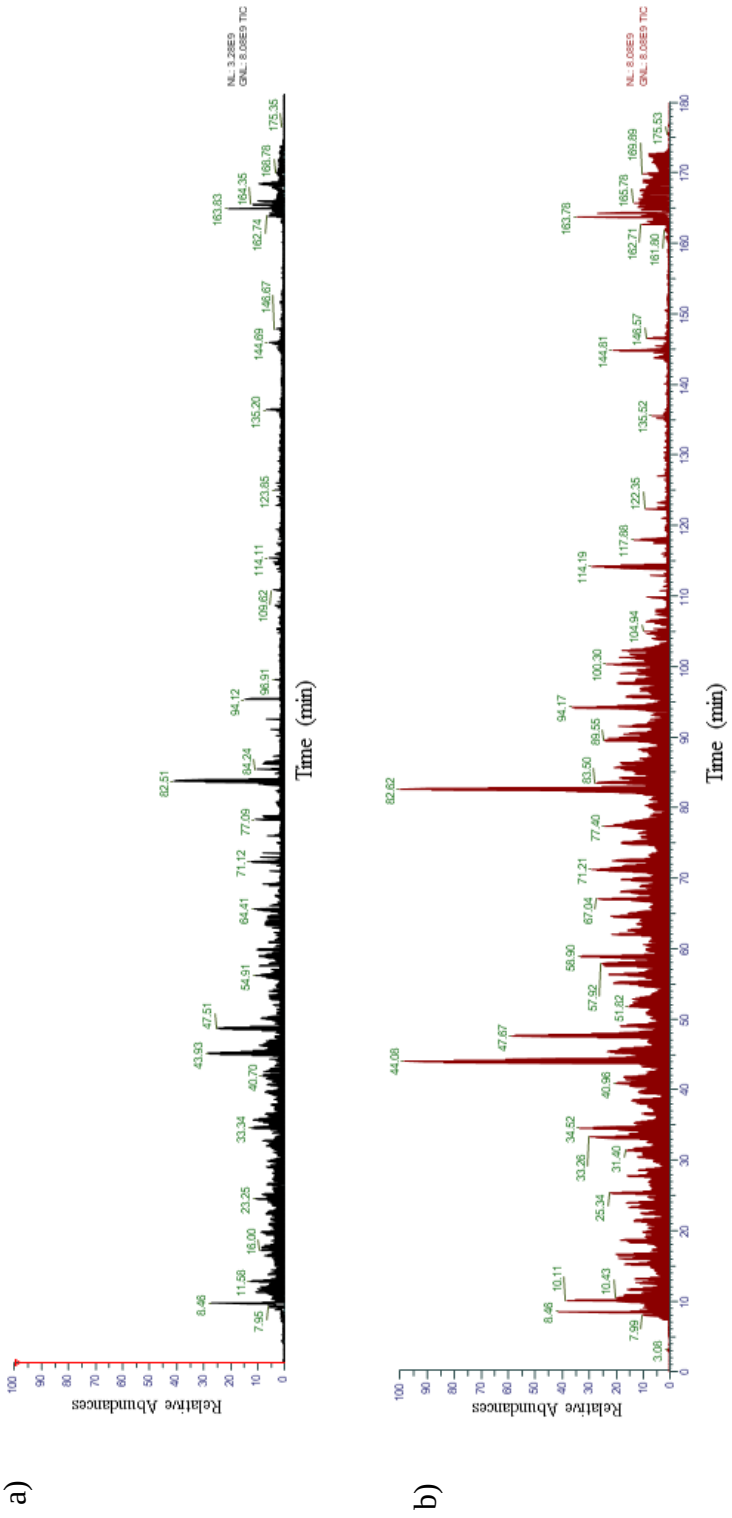
b)



P67910	P67910	3	3	3	ADP-L-gly	hldD	sp|P6791	1	3	3	3	12.3	12.3	12.3	34.893	310	310	0	24.626	23719000	3	AAGYDKPFK;AESFQAVADATLAYHK;EYEKPI	473	11;52;453	True;True	11;56;497	17;83;614	43;126;885	43;126;885			-1
P00968	P00968	8	8	8	Carbamoy	carB	sp|P00968	1	8	8	8	13.4	13.4	13.4	117.84	1073	1073	0	95.705	23562000	9	AMEIVYDEADLRR;EIGYPLVVRPSYVLGGR;IG	28	129;378;78	True;True	141;142;4	176;177;5	232;233;7	233;777;16	38;39	725;1068	-1
P77338	P77338	4	4	4	Mechanos	mscK	sp|P77338	1	4	4	4	5.2	5.2	5.2	127.21	1120	1120	0	45.515	23515000	4	IKEETVQLR;LEHQLQLLQEAVNSK;VLMQAQ	511	809;998;17	True;True	876;1082;1	1104;1340	1657;1967	1657;1967;3345;3434			-1
P0ACF8	P0ACF8	2	2	2	DNA-bind	hns	sp|P0ACF	1	2	2	2	25.5	25.5	25.5	15.539	137	137	0	17.283	23441000	4	EMLIADGIDPNELLNSLAAVK;REEESAAAAEV	245	411;1412	True;True	452;453;1	559;560;1	820;821;2	820;2821	205	64	-1
P77398	P77398	6	6	6	Bifunctior	arnA	sp|P77398	1	6	6	6	14.8	14.8	14.8	74.288	660	660	0	128.48	23430000	7	AITQLILNLVEGSPIK;APLNWVLVNGETETGV	512	98;147;337	True;True	107;163;37	142;202;4	192;276;67	192;276;67	321	638	-1
P0A8U2	P0A8U2	3	3	3	UPF0294 p	yafD	sp|P0A8U	1	3	3	3	16.2	16.2	16.2	29.991	266	266	0	24.811	23345000	3	AFGRPLDFVFYR;ILPPGSFASIGQALPPGEPLS	159	61;828;11	True;True	66;897;13	95;1133;1	139;1718;2	139;1718;2386			-1
P38054	P38054	8	8	8	Cation eff	cusA	sp|P38054	1	8	8	8	14.5	14.5	14.5	114.71	1047	1047	0	119.92	23336000	9	DRDMVSVVHDLQK;IDMLSTGIK;LEEWQHQI	428	287;752;9	True;True	320;817;1	390;1035;	544;1562;1	544;1562;1963;2191;3048;3184;3			-1
P0A7S3	P0A7S3	2	2	2	30S ribosc	rpsL	sp|P0A7S	1	2	2	2	32.3	32.3	32.3	13.737	124	124	0	72.914	23095000	3	LTNGFEVTSYIGGEGHNLQEHSVILIR;SNVPAI	125	1141;1514	True;True	1236;1670	1563;1564	2278;2279	2278;2978			-1
P64604	P64604	4	4	4	Probable	mlaD	sp|P64604	1	4	4	4	43.7	43.7	43.7	19.576	183	183	0	27.932	22911000	6	NSGDAPAAAPGNNETTEPVGTTK;SAMVLED	471	1282;1454	True;True	1405;1605	1767;2025	2542;2543	2543;2895	313	141	-1
P0A836	P0A836	4	4	4	Succinyl-C	sucC	sp|P0A83	1	4	4	4	16	16	16	41.392	388	388	0	35.13	22808000	4	CDLIADGIIGAVAEVGVNVPVVVR;IILSDDKV	142	216;804;1	True;True	239;870;1	293;1092;	425;1626;2	425;1626;2076;3219			-1
P0A7X3	P0A7X3	3	3	3	30S ribosc	rpsI	sp|P0A7X	1	3	3	3	26.9	26.9	26.9	14.856	130	130	0	20.355	22371000	3	ALMEYDESLRSELR;SLEQYFGRETAR;VFIKPGI	135	119;1502;1	True;True	130;1657;1	165;2085;	220;2960;3	220;2960;3	116	88	-1
P0A7N4	P0A7N4	1	1	1	50S ribosc	rpmF	sp|P0A7N	1	1	1	1	17.5	17.5	17.5	6.4463	57	57	0	13.136	22050000	1	HHITADGYYR	120	693	TRUE	754	946	1402	1402			-1
P0ABK5	P0ABK5	3	3	3	Cysteine s	cysK	sp|P0ABK	1	3	3	3	18.6	18.6	18.6	34.489	323	323	0	37.227	21998000	4	GVLKPGVELVEPTSGNTGIALAYVAAAR;VIGI	232	659;1724;1	True;True	713;1897;2	886;2359;2	1269;1270	1270;3303;3608			-1
P0A7L8	P0A7L8	3	3	3	50S ribosc	rpmA	sp|P0A7L	1	3	3	3	30.6	30.6	30.6	9.1244	85	85	0	25.539	21950000	3	FGGESVLAGSIIVR;FHAGANVGCGR;RFGGES	116	480;491;1	True;True	524;536;1	641;655;1	917;938;2	917;938;2825			-1
P25714	P25714	3	3	3	Membran	yidC	sp|P2571	1	3	3	3	10.6	10.6	10.6	61.525	548	548	0	71.174	21763000	5	ELNSTQPFQLLETSPQFIYQAQSGLTGR;GAAY	374	402;541;1	True;True	442;587;2	546;547;7	804;805;8	805;1009;3602			-1
P0AC38	P0AC38	2	2	2	Aspartate	aspA	sp|P0AC3	1	2	2	2	7.3	7.3	7.3	52.356	478	478	0	15.269	21737000	3	AGLNEINLPELQAGSSIMPAK;EVPADAYYGV	241	74;448	True;True	81;491	113;607	160;876;87	160;877	202	324	-1
P0DTT0	P0DTT0	5	5	5			sp|P0DTT	1	5	5	5	14	14	14	67.355	607	607	0	51.749	21690000	6	ELVHNVALR;INIVDTPGHADFGGEVER;LFLG	325	407;838;1	True;True	447;910;1	552;1150;	812;1743;1	812;1743;1	260	91	-1
P0AGJ5	P0AGJ5	4	4	4	Uncharact	yfiF	sp|P0AGJ	1	4	4	4	22.9	22.9	22.9	37.784	345	345	0	52.892	21514000	4	GVVVQDAALLESGAAIR;MVLVLGQEYEGLPD	316	666;1218;1	True;True	720;1331;1	895;1671;	1281;2419	1281;2419;2663;3506			-1
P77211	P77211	1	1	1	Cation eff	cusC	sp|P7721	1	1	1	1	3.9	3.9	3.9	50.269	457	457	0	18.793	20561000	1	ALYQHGAVSYLEVLDAER	509	127	TRUE	139	174	230	230			-1
P68187	P68187	2	2	2	Maltose/r	malK	sp|P68187	1	2	2	2	8.9	8.9	8.9	40.99	371	371	0	54.651	20133000	3	VAQVGKPLELYHYPADR;VNQVAEVLQLAHLI	474	1668;1772	True;True	1836;1951	2292;2293	3227;3228	3227;3381			-1
P31057	P31057	2	2	2	3-methyl-	panB	sp|P3105	1	2	2	2	13.6	13.6	13.6	28.237	264	264	0	87.931	20065000	3	IEGGEWLVETVQMLTER;QYMAEVESGVYPGI	393	762;1402	True;True	827;1545;1	1045;1953	1573;2802	1573;2803	281	248	-1
P0A8M6	P0A8M6	2	2	2	UPF0265 p	yeeX	sp|P0A8M	1	2	2	2	37.6	37.6	37.6	12.778	109	109	0	48.444	19815000	5	GDYEDRVDDYIIK;VLLLDNLSDYIKPGMSVEAI	154	560;1751	True;True	608;1925;1	742;2391;2	1034;3340	1034;3341	118;119	56;68	-1
P0AC02	P0AC02	2	2	2	Outer mei	bamD	sp|P0AC0	1	2	2	2	9.8	9.8	9.8	27.829	245	245	0	29.3	19637000	2	GAWVAVVNR;LNPTHPNIDYVMYMR	238	554;1095	True;True	602;1188	735;1504	1027;2183	1027;2183			-1
P0A717	P0A717	3	3	3	Ribose-ph	prs	sp|P0A71	1	3	3	3	16.2	16.2	16.2	34.218	315	315	0	52.2	19185000	3	ANVSQVMHIIGDVAGR;IANRLYTSLGDAAV	101	143;741;1	True;True	159;805;1	198;1005;2	272;1470;3	272;1470;3256			-1
P0AB67	P0AB67	2	2	2	NAD(P) tr	pntB	sp|P0AB6	1	2	2	2	8	8	8	48.722	462	462	0	36.486	19050000	6	FGIHPVAGR;NSHSVIITPGYGMAVAQAQYP	213	481;1283	True;True	525;1406;1	642;1768;1	918;2544;2	918;2544	172	317	-1
P0A9B2	P0A9B2	4	4	4	Glyceralde	gapA	sp|P0A9B	1	4	4	4	21.8	21.8	21.8	35.532	331	331	0	59.211	18889000	4	VINDNFGIIEGLMTTVHATTATQK;VPTPNVS	178	1729;1780	True;True	1902;1960	2364;2440	3309;3418	3309;3418;3460;3512			-1
P15034	P15034	3	3	3	Xaa-Pro ai	pepP	sp|P1503	1	3	3	3	10.2	10.2	10.2	49.815	441	441	0	51.35	18563000	3	EIYDIVLESLETSLR;LYRPGTSILEVTGEVVR;SD	333	382;1164;1	True;True	421;1262;1	523;1596;2	781;2324;2	781;2324;2898			-1
P0AEN8	P0AEN8	2	2	2	L-fucose r	fucU	sp|P0AEN	1	2	2	2	27.9	27.9	27.9	15.473	140	140	0	102.08	18220000	2	AFAIVITGER;VLAEMGHGDEIIFSDAHFPAHS	275	58;1742	True;True	63;1915	92;2378	136;3325	136;3325			-1
P0ABD5	P0ABD5	2	2	2	Acetyl-co	accA	sp|P0ABD	1	2	2	2	7.2	7.2	7.2	35.241	319	319	0	19.265	18077000	2	HPQRPYTLDYVR;LDINIDEEVHR	224	711;980	True;True	772;1062	965;1318	1427;1942	1427;1942			-1
P21599	P21599	4	4	4	Pyruvate l	pykA	sp|P2159	1	4	4	4	21.5	21.5	21.5	51.357	480	480	0	87.911	17932000	12	AEAVCSQDAMDDIILASDVVMVAR;AEVMD	352	42;55;887;	True;True	44;59;60;9	69;86;87;8	108;129;1	108;132;1	274;275;2	302;367;3	-1
P32170	P32170	3	3	3	L-rhamno	rhaA	sp|P3217	1	3	3	3	8.1	8.1	8.1	47.199	419	419	0	24.246	17120000	3	DQIKPEHFK;KLEAAGDYTAR;VHIGLDFFDASI	402	283;928;1	True;True	315;1008;1	385;1255;2	536;1869;3	536;1869;3285			-1
P0AGE9	P0AGE9	3	3	3	Succinyl-C	sucD	sp|P0AGE	1	3	3	3	16.6	16.6	16.6	29.777	289	289	0	68.947	16821000	5	GGTTHLGLPVFNTVR;VICQGFTGSQGTFHSE	314	589;1719;1	True;True	638;1892;1	778;2354;2	1122;3296	1122;3298;3321			-1
P21156	P21156	1	1	1	Sulfate ad	cysD	sp|P2115	1	1	1	1	3.6	3.6	3.6	35.188	302	302	0	7.3188	16735000	1	QLEAESIHIIR	346	1364	TRUE	1502	1892	2733	2733			-1
P07395	P07395	3	3	3	Phenylala	pheT	sp|P0739	1	3	3	3	8.1	8.1	8.1	87.377	795	795	0	38.178	16599000	5	DVAVLNQLPLVQPEIVPVGATIDDTLPITVEAI	53	303;780;1	True;True	336;845;1	413;414;1	594;595;5	595;1594;2177			-1
P77718	P77718	3	3	3	tRNA sulfu	thiI	sp|P7771	1	3	3	3	8.3	8.3	8.3	54.972	482	482	0	27.835	16521000	4	EHIINLAR;VHYCFFNLGGAAHEIGVR;YVGGG	516	365;1718;1	True;True	400;1891;2	498;2353;2	741;742;3	741;3295;3736			-1
P0ABU0	P0ABU0	1	1	1	1,4-dihydr	menB	sp|P0ABU	1	1	1	1	3.2	3.2	3.2	31.633	285	285	0	6.385	16087000	1	ITINRPQVR	235	869	TRUE	944	1184	1784	1784			-1
P0AC53	P0AC53	1	1	1	Glucose-6	zwf	sp|P0AC5	1	1	1	1	4.1	4.1	4.1	55.704	491	491	0	69.397	15906000	1	LDLSYSETFNQTHLADAYER	244	983	TRUE	1065	1321	1945	1945			-1
P0AA25	P0AA25	1	1	1	Thioredox	trxA	sp|P0AA2	1	1	1	1	13.8	13.8	13.8	11.806	109	109	0	12.146	15167000	1	IIHLTDDSFDTDVLK	200	802	TRUE	868	1090	1624	1624			-1
P76558	P76558	3	3	3	NADP-dep	maeB	sp|P7655	1	3	3	3	6.5	6.5	6.5	82.416	759	759	0	31.617	14985000	4	IQVSPTKPLATQR;IVNMVALAVVEAQTQPL;	505	853;882;1	True;True	927;958;1	1167;1201	1763;1802	1763;1802;3223			-1
P23830	P23830	4	4	4	CDP-diacy	pssA	sp|P2383	1	4	4	4	11.1	11.1	11.1	52.801	451	451	0	28.405	14943000	5	DAAYHFQGDADNDQLSVTPLVGLGK;DDDN	361	221;233;9	True;True	247;260;1	303;323;1	436;462;1	436;462;1938;2773			-1
P24182	P24182	1	1	1	Biotin carl	accC	sp|P2418	1	1	1	1	4	4	4	49.32	449	449	0	14.677	14937000	2	IQVEHPVTEMITGVDLIK	367	852	TRUE	925;926	1165;1166	1761;1762	1761	277	302	-1
Q47270	Q47270	1	1	1	Protein Ni	ninE	sp|Q4727	1	1	1	1	10.7	10.7	10.7	6.4937	56	56	0.009615	5.439	14819000	1	ATPLIR	519	178	TRUE	197	238	320	320			-1
P69222	P69222	2	2	2	Translatio	infA	sp|P6922	1	2	2	2	52.8	52.8	52.8	8.2495	72	72	0	15.957	14303000	4	AKEDNIEMQGTVLETLPNTMFR;VELENGHV	480	102;1685	True;True	111;112;1	146;147;2	198;199;2	198;3252	315;316	9;21	-1
P0AG90	P0AG90	2	2	2	Protein tr	secD	sp|P0AG9	1	2	2	2	4.4	4.4	4.4	66.631	615	615	0	18.97	13777000	3	HPDLVISSQGSNQLR;KENNYGLSITFR	310	708;911	True;True	769;990	961;1235	1419;1420	1419;1844			-1
P08660	P08660	2	2	2	Lysine-ser	lysC	sp|P0866	1	2	2	2	8.2	8.2	8.2	48.531	449	449	0	35.661	13717000	3	AEPDIAALAELAALQLLPR;GGSDYTAALLAEA	63	49;583	True;True	52;632	78;771	120;121;1	121;1111			-1
P0AFI5	P0AFI5	1	1	1	D-alanyl-[	pbpG	sp|P0AFI	1	1	1	1	5.2	5.2	5.2	33.887	310	310	0	12.521	13553000	1	FVEPTGLSVHNVSTAR	293	533	TRUE	578	707	999	999			-1
P0A858	P0A858	1	1	1	Triosepho	tpiA	sp|P0A85	1	1	1	1	5.5	5.5	5.5	26.972	255	255	0	9.0479	13285000	1	DIGAQYIIIGHSER	146	259	TRUE	287	352	497	497			-1
P0AGD3	P0AGD3	1	1	1	Superoxid	sodB	sp|P0AGD	1	1	1	1	9.3	9.3	9.3	21.265	193	193	0	18.55	13041000	2	DALAPHISAETIEYHYGK	312	226	TRUE	253	309;310	443;444	444			-1
P14081	P14081	1	1	1	Selenocys	selB	sp|P1408	1	1	1	1	2.3	2.3	2.3	68.867	614	614	0	12.026	12985000	1	AQSDADALSVHLER	332	157	TRUE	175	214	291	291			-1
P26616	P26616	3	3	3	NAD-depe	maeA	sp|P2661	1	3	3	3	8.3	8.3	8.3	63.197	565	565	0	154.59	12596000	4	ENLSDWDTDSDVLSLLDVVR;GVFISYQNR;IT	378	419;654;8	True;True	461;708;9	568;874;1	829;1252;1	829;1252;1778			-1
P69411	P69411	2	2	2	Outer mei	rcsF	sp|P6941	1	2	2	2	29.1	29.1	29.1	14.163	134	134	0	21.111	12544000	2	DLGEVSGDSCQASNQDSPPSIPTAR;IYTNAEE	481	270;894	True;True	299;971	365;1216	511;1822	511;1822			-1
P0AG63	P0AG63	1	1	1	30S ribosc	rpsQ	sp|P0AG6	1	1	1	1	22.6	22.6	22.6	9.7043	84	84	0	19.643	12472000	2	LHVHDENNECGIGDVVEIR	307	1041	TRUE	1129	1427;1428	2085;2086	2086			-1
P77757	P77757	2	2	2	Undecapr	arnC	sp|P7775	1	2	2	2	7.1	7.1	7.1	36.339	322	322	0	14.93	12383000	3	AIEIPVHHAER;HIVDAMLHCHER	518	91;698	True;True	100;759	134;135;9	183;184;1	184;1408			-1
P0AEK4	P0AEK4	2	2	2	Enoyl-[acy	fabI	sp|P0AEK	1	2	2	2	10.7	10.7	10.7	27.864	262	262	0	24.236	12365000	2	IAHDISSYSFVAMAK;LSIAYGIAQAMHR	274	738;1129	True;True	802;1224	1002;1550	1466;2263	1466;2263			-1
P30958	P30958	4	4	4	Transcript	mfd	sp|P3095	1	4	4	4	5.7	5.7	5.7	129.98	1148	1148	0	56.932	12363000	4	LEAIASLEDLGAGFALATHDLEIR;LYVPVSSLHI	392	993;1166;1	True;True	1077;1264	1334;1598	1961;2326	1961;2326;2786;3093			-1
P09546	P09546	3	3	3	Bifunctior	putA	sp|P0954	1	3	3	3	3.2	3.2	3.2	143.81	1320	1320	0	27.151	12301000	3	EAVDFLHYYAGQVR;EVFGPVLHVVR;IYAPV	68	332;446;8	True;True	365;489;9	457;605;1	665;873;1	665;873;1813			-1
P76108	P76108	1	1	1	Putative A	ydcS	sp|P7610	1	1	1	1	2.1	2.1	2.1	42.295	381	381	0.009597	5.4384	12240000	1	TLDPRVVK	494	1597	TRUE	1759	2199	3102	3102			-1
P22523	P22523	5	5	5	Chromosc	mukB	sp|P2252	1	5	5	5	4.8	4.8	4.8	170.23	1486	1486	0	56.822	12198000	5	FIGSHLAVAFESDPEAEIR;IESLHAER;LAVADI	357	499;769;9	True;True	544;834;1	663;1052;1	947;1581;1	947;1581;1929;2182;3035			-1
P76372	P76372	2	2	2	Chain leng	wzzB	sp|P7637	1	2	2	2	6.7	6.7	6.7	36.454	326	326	0	12.257	12152000	2	PLVFSPNYYQTR;VDDLDIHAYR	500	1314;1677	True;True	1447;1846	1829;2303	2643;3238	2643;3238			-1
P0A953	P0A953	2	2	2	3-oxoacyl-	fabB	sp|P0A95	1	2	2	2	3.7	3.7	3.7	42.613	406	406	0	10.829	11724000	2	ELAAIR;LDTTGLIDR	173	391;989	True;True	430;1071	533;1327	791;1952	791;1952			-1
P33643	P33643	3	3	3	Ribosoma	rluD	sp|P3364	1	3	3	3	16.6	16.6	16.6	37.121	326	326	0	57.718	11367000	3	EYEAVAIGHMTAGGTVDEPISR;LDKDTTGLM	410	452;981;1	True;True	496;1063;1	613;1319;2	884;1943;3	884;1943;3	299	179	-1
P00350	P00350	3	3	3	6-phosph	gnd	sp|P0035	1	3	3	3	13	13	13	51.481	468	468	0	59.79	11364000	3	EKTEEVIAENPGK;IAAVAEDGEPCVTYIGADG	12	390;731;1	True;True	429;795;2	532;994;2	790;1457;3	790;1457;3545			-1
P0ADY1	P0ADY1	2	2	2	Peptidyl-[	ppiD	sp|P0ADY	1	2	2	2	8.7	8.7	8.7	68.149	623	623	0	112	11351000	4	AMVQGITQNNAQIVFEALMSNLR;LDAATM	261	134;974	True;True	150;1056	189;1310;1	260;1932;1	260;1932	208	588	-1
P25888	P25888	2	2	2	ATP-depe	rhlE	sp|P2588	1	2	2	2	5.9	5.9	5.9	49.989	454	454	0	25.29	11309000	2	MLDMGFIHDIR;NTASDQVTQHVHFVDK	377	1188;1286	True;True	1294;1413	1633;1778	2373;2565	2373;2565			-1
P77330	P77330	1	1	1	Lipoprote	borD	sp|P7733	1	1	1	1	15.5	15.5	15.5	10.447	97	97	0	7.8061	11308000	2	ETITHHFFVSGIGQK	510	439	TRUE	482	598	865;866	866			-1
P0ABA0	P0ABA0	2	2	2	ATP synth	atpF	sp|P0ABA	1	2	2	2	16.7	16.7	16.7	17.264	156	156	0	16.115	10930000	2	IVAQAQAEIEAER;QKEIADGLASAER	216	873;1361	True;True	949;1499	1189;1888	1789;2727	1789;2727			-1
P75876	P75876	3	3	3	Ribosoma	rlmI	sp|P7587	1	3	3	3	9.3	9.3	9.3	44.356	396	396	0	24.456	10832000	3	ASLGETIDIVDHQGK;LLVDIQHGHK;RHPWVI	493	168;1081;1	True;True	186;1171;1	226;1473;1	304;2144;2	304;2144;2835			-1
P36929	P36929	2	2	2	Ribosoma	rsmB	sp|P3692	1	2	2	2	7.9	7.9	7.9	48.347	429	429	0	22.689	10822000	2	GFPHADYPDAVR;TTHILEVAPEAQVVAVDID	415	573;1639	True;True	622;1806	761;2260	1098;3194	1098;3194			-1
P0AG51	P0AG51	1	1	1	50S ribosc	rpmD	sp|P0AG5	1	1	1	1	23.7	23.7	23.7	6.5417	59	59	0	8.867	10794000	2	IGHTVEREDTPAIR	304	783	TRUE	848	1067;1068	1597;1598	1597			-1
P0A6Y1	P0A6Y1	1	1	1	Integratio	ihfB	sp|P0A6Y	1	1	1	1	11.7	11.7	11.7	10.651	94	94	0	6.083	10630000	1	LATQQSHIPAK	95	970	TRUE	1052	1306	1928	1928			-1
P27306	P27306	2	2	2	Soluble py	sthA	sp|P2730	1	2	2	2	6	6	6	51.56	466	466	0	20.605	10600000	2	IIEFNQNPLYSDHSR;PYHPTDVDFTHPR	381	799;1323	True;True	865;1456	1087;1839	1620;2658	1620;2658			-1
P0AFC7	P0AFC7	2	2	2	NADH-qui	nuoB	sp|P0AFC	1	2	2	2	12.7	12.7	12.7	25.056	220	220	0	18.064	10530000	2	IDPNGENDRYPLQK;RPLSWVVGDQGVYR	284	755;1437	True;True	820;1584	1038;1998	1565;2856	1565;2856			-1
P00582	P00582	5	5	5	DNA polyi	polA	sp|P0058	1	5	5	5	9.5	9.5	9.5	103.12	928	928	0	52.292	10439000	6	ALELLKPLLEDEK;AMGLPLLAVSGVEADDVIG	19	113;130;5	True;True	123;143;6	158;178;7	212;234;1	212;234;1	25;26	102;443	-1
P0A927	P0A927	3	3	3	Nucleosid	tsx	sp|P0A92	1	3	3	3	18	18	18	33.589	294	294	0	23.736	10365000	3	GIWNHGSPLFMEIEPR;LTNTDLSFGPFK;TNN	169	603;1142;1	True;True	653;1237;1	794;1565;2	1146;2280	1146;2280;3133			-1
P0ABS1	P0ABS1	1	1	1	RNA polyr	dksA	sp|P0ABS	1	1	1	1	11.9	11.9	11.9	17.528	151	151	0	13.467	10239000	2	TVTHMQDEAANFPDPVDR	233	1651	TRUE	1818;1819	2274;2275	3209;3210	3210	198	62	-1
P39099	P39099	1	1	1	Periplasm	degQ	sp|P3909	1	1	1	1	3.1	3.1	3.1	47.204	455	455	0	17.018	10237000	1	TLAQQLIDFGEIKR	429	1594	TRUE	1756	2196	3099	3099			-1
P06992	P06992	2	2	2	Ribosoma	rsmA	sp|P0699	1	2	2	2	12.1	12.1	12.1	30.42	273	273	0	14.06	9940000	3	FGQNFLNDQFVIDSIVSAINPQK;LQTHPFLGI	46	484;1118	True;True	528;1213	645;1538	921;922;2	921;2249			-1
P08839	P08839	2	2	2	Phosphoe	ptsI	sp|P0883	1	2	2	2	4.2	4.2	4.2	63.561	575	575	0	11.413	9894000	2	ALLLKEDEIVIDR;QVIDASHAEGK	64	117;1394	True;True	127;1536	162;1942	217;2789	217;2789			-1
P07813	P07813	3	3	3	Leucine--t	leuS	sp|P0781	1	3	3	3	3.6	3.6	3.6	97.233	860	860	0	18.18	9876400	3	AAENNPELAAFIDECR;FFTELYK;LRDWGVSR	55	2;475;112	True;True	2;519;121	4;636;154	5;911;225	5;911;2252			-1
P0AG44	P0AG44	1	1	1	50S ribosc	rplQ	sp|P0AG4	1	1	1	1	7.1	7.1	7.1	14.364	127	127	0	7.6357	9760300	1	TRDNEIVAK	303	1629	TRUE	1795	2246	3175	3175			-1
P0A6H1	P0A6H1	3	3	3	ATP-depe	clpX	sp|P0A6H	1	3	3	3	14.2	14.2	14.2	46.355	424	424	0	37.887	9577000	3	LLDVPFTMADATTLTEAGYVGEDVENIIQK;LP	82	1068;1107	True;True	1158;1201	1459;1525	2128;2226	2128;2226;2888			-1
P0A9S3	P0A9S3	2	2	2	Galactitol	gatD	sp|P0A9S	1	2	2	2	3.2	3.2	3.2	37.39	346	346	0	16.347	9541900	2	KLSLEPLIAHR;LSLEPLIAHR	194	930;1131	True;True	1010;1226	1257;1552	1871;2265	1871;2265			-1
P0ADW3	P0ADW3	1	1	1	Inner mer	yhcB	sp|P0ADV	1	1	1	1	9.8	9.8	9.8	14.961	132	132	0	16.408	9535600	2	SAELLDTMAHDYR	260	1449	TRUE	1597;1598	2017;2018	2885;2886	2886	207	66	-1
P0AGD7	P0AGD7	5	5	5	Signal rec	ffh	sp|P0AGD	1	5	5	5	17	17	17	49.787	453	453	0	33.772	9429900	5	ILGMGDVLSLIEDIESK;MFDNLTDR;NELVAAI	313	821;1174;1	True;True	889;1274;1	1119;1611	1675;2343	1675;2343	248	1	-1
P0AAF6	P0AAF6	2	2	2	Arginine t	artP	sp|P0AAF	1	2	2	2	21.1	21.1	21.1	27.022	242	242	0	35.868	9313400	3	ALMMEPQVLLFDEPTAALDPEITAQIVSIIR;EL	205	120;393	True;True	131;432	166;535	221;222;7	221;793	161;162	157;158	-1
P0A825	P0A825	1	1	1	Serine hyc	glyA	sp|P0A82	1	1	1	1	4.3	4.3	4.3	45.316	417	417	0	10.105	9183600	1	VVSGGTDNHLFLVDLVDK	141	1817	TRUE	2002	2494	3491	3491			-1
P0ABU9	P0ABU9	1	1	1	Protein Tc	tolQ	sp|P0ABU	1	1	1	1	6.5	6.5	6.5	25.597	230	230	0	7.3351	9151500	2	ANSHAPEAVVEGASR	237	142	TRUE	158	197	270;271	271			-1
P11447	P11447	3	3	3	Argininos	argH	sp|P1144	1	3	3	3	13.6	13.6	13.6	50.318	457	457	0	55.775	9141800	3	ARPQQILESDAEDIHSWVEGK;EAHHIVGEAV	327	163;324;1	True;True	181;357;1	221;447;1	298;655;2	298;655;2757			-1
P60906	P60906	2	2	2	Histidine-	hisS	sp|P6090	1	2	2	2	6.1	6.1	6.1	47.029	424	424	0	23.235	8993600	2	LRDELPGVK;SGEQTAVAQDSVAAHLR	461	1120;1478	True;True	1215;1632	1540;2057	2251;2928	2251;2928			-1
P0A9K3	P0A9K3	3	3	3	PhoH-like	ybeZ	sp|P0A9K	1	3	3	3	10.1	10.1	10.1	39.038	346	346	0	17.566	8990200	3	ILLTRPAVEAGEK;LGIEINRR;TYLAVAAAVDA	186	826;1020;1	True;True	894;1106;1	1130;1378	1714;2034	1714;2034;3212			-1
P75818	P75818	1	1	1	Uncharact	ybjP	sp|P7581	1	1	1	1	10.5	10.5	10.5	18.991	171	171	0	13.372	8965800	1	TTLPDSAHVASASTIPNR	491	1640	TRUE	1807	2261	3195	3195			-1
P0AES4	P0AES4	2	2	2	DNA gyras	gyrA	sp|P0AES	1	2	2	2	3.4	3.4	3.4	96.962	875	875	0	12.515	8877300	2	ETIIVHEIPYQVNK;LLVANTHDHILCFSSR	277	438;1080	True;True	481;1170	597;1472	864;2143	864;2143			-1
P61517	P61517	1	1	1	Carbonic a	can	sp|P6151	1	1	1	1	5.9	5.9	5.9	25.096	220	220	0	11.854	8611900	1	LTGLEPGELFVHR	464	1139	TRUE	1234	1561	2276	2276			-1
P0A6P7	P0A6P7	2	2	2	Probable	engB	sp|P0A6P	1	2	2	2	15.7	15.7	15.7	23.561	210	210	0	17.995	8601000	2	HLPSDTGIEVAFAGR;LVDLPGYGYAEVPEEMI	89	703;1150	True;True	764;1246	956;1580	1413;2306	1413;2306			-1
P0A7T7	P0A7T7	1	1	1	30S ribosc	rpsR	sp|P0A7T	1	1	1	1	16	16	16	8.9863	75	75	0	6.015	8538400	1	YLSLLPYTDRHQ	128	1883	TRUE	2072	2587	3609	3609			-1
P23869	P23869	1	1	1	Peptidyl-p	ppiB	sp|P2386	1	1	1	1	6.7	6.7	6.7	18.153	164	164	0	5.6725	8487800	1	EGFYNNTIFHR	365	354	TRUE	387	479	689	689			-1
P0A800	P0A800	1	1	1	DNA-direc	rpoZ	sp|P0A80	1	1	1	1	25.3	25.3	25.3	10.236	91	91	0	73.548	8451400	1	ERQEQQEQEAAELQAVTAIAEGR	137	431	TRUE	474	582	846	846			-1
P23865	P23865	2	2	2	Tail-speci	prc	sp|P2386	1	2	2	2	4.4	4.4	4.4	76.662	682	682	0	17.248	8450200	2	EIDPHTNYLSPR;FSASASEIFAAAMQDYGR	364	372;521	True;True	409;566	507;693	751;983	751;983			-1
P0A6T5	P0A6T5	1	1	1	GTP cyclol	folE	sp|P0A6T	1	1	1	1	6.3	6.3	6.3	24.83	222	222	0	16.501	8411700	1	IVQFFAQRPQVQER	93	884	TRUE	960	1203	1805	1805			-1
P31224	P31224	3	3	3	Multidrug	acrB	sp|P3122	1	3	3	3	6.7	6.7	6.7	113.57	1049	1049	0	23.043	8324100	4	AQALGVSINDINTTLGAAWGGSYVNDFIDR;L	395	152;1106;1	True;True	169;1200;1	208;1524;2	282;283;2	282;2225;2990			-1
P0ABH0	P0ABH0	2	2	2	Cell divisi	ftsA	sp|P0ABH	1	2	2	2	11.4	11.4	11.4	45.329	420	420	0	26.885	8310700	3	IGAPLNITGLTDYAQEPYYSTAVGLLHYGK;VLH	226	777;1749	True;True	842;1923	1061;2389	1591;3337	1591;3338			-1
P0AA91	P0AA91	1	1	1	Uncharact	yeaY	sp|P0AA9	1	1	1	1	10.9	10.9	10.9	20.921	193	193	0	19.898	8175700	1	LEIATVPLDSGARPTLGEPSR	202	999	TRUE	1083	1341	1968	1968			-1
P39285	P39285	4	4	4	Minicondi	mscM	sp|P3928	1	4	4	4	7.2	7.2	7.2	123.97	1107	1107	0	73.419	8104200	7	ELLNSLLQGGDTLLLELTK;PAQPEVVEALQSAI	431	399;1300;1	True;True	438;1430;1	542;1807;1	800;2614;2	800;2614;2721;3009			-1
P32176	P32176	3	3	3	Formate d	fdoG	sp|P3217	1	3	3	3	5.7	5.7	5.7	112.55	1016	1016	0	32.791	7969000	3	ASIFHIEGDPDHPVNR;GADAPGIALTDGEILS	403	165;542;1	True;True	183;588;1	223;718;1	300;1010;2	300;1010;2436			-1
P60723	P60723	2	2	2	50S ribosc	rplD	sp|P6072	1	2	2	2	13.4	13.4	13.4	22.086	201	201	0	23.156	7798100	2	SGGVTFAARPQDHSQK;SILSELVRQDR	458	1483;1492	True;True	1637;1646	2062;2072	2933;2945	2933;2945			-1
P00509	P00509	2	2	2	Aspartate	aspC	sp|P0050	1	2	2	2	11.4	11.4	11.4	43.573	396	396	0	22.525	7785400	3	ANYSNPPAHGASVVATILSNDALR;MFENITA	15	144;1175	True;True	160;1275;1	199;1612;1	273;2344;2	273;2344	23	1	-1
P39407	P39407	1	1	1	Uncharact	yjjU	sp|P3940	1	1	1	1	6.2	6.2	6.2	39.831	357	357	0	11.56	7686100	1	WLGDSSLQPLVNLVQHHETSYR	434	1840	TRUE	2026	2521	3519	3519			-1
P39177	P39177	3	3	3	Universal	uspG	sp|P3917	1	3	3	3	36.6	36.6	36.6	15.935	142	142	0	42.476	7617000	4	DEVNELAEELGADVVVIGSR;LQTMVSHFTIDI	430	240;1119;1	True;True	267;1214;1	331;1539;1	471;472;2	472;2250;2537			-1
P0A8W8	P0A8W8	2	2	2	UPF0304 p	yfbU	sp|P0A8W	1	2	2	2	14.6	14.6	14.6	19.536	164	164	0	19.661	7500200	2	MLNVWHACPR;RVTFLGFDAATEAR	161	1191;1442	True;True	1298;1590	1637;2006	2377;2871	2377;2871			-1
P0A9V1	P0A9V1	3	3	3	Lipopolys	lptB	sp|P0A9V	1	3	3	3	17.4	17.4	17.4	26.8	241	241	0	22.556	7443800	3	ANELMEEFHIEHLR;DSGLGVLITDHNVR;LSVY	195	139;295;1	True;True	155;328;1	194;401;1	266;580;2	266;580;2272			-1
P0A9J0	P0A9J0	2	2	2	Ribonucle	rng	sp|P0A9J	1	2	2	2	6.5	6.5	6.5	55.364	489	489	0	28.771	7364900	2	VAYIDGGILQEIHIER;YLVFMPGASHVGVSQR	185	1673;1885	True;True	1842;2074	2299;2589	3234;3611	3234;3611			-1
P0A9C0	P0A9C0	1	1	1	Anaerobic	glpA	sp|P0A9C	1	1	1	1	1.8	1.8	1.8	58.958	542	542	0	12.828	7358500	2	NHGLLHSGAR	179	1254	TRUE	1373	1726	2491;2492	2491			-1
P0A6G7	P0A6G7	1	1	1	ATP-depe	clpP	sp|P0A6G	1	1	1	1	10.6	10.6	10.6	23.186	207	207	0	16.117	7355900	2	VMIHQPLGGYQGQATDIEIHAR	81	1763	TRUE	1941	2411	3366;3367	3366			-1
P0A6A0	P0A6A0	2	2	2	Probable	ubiB	sp|P0A6A	1	2	2	2	3.5	3.5	3.5	63.202	546	546	0	17.752	7136600	3	LRPTEVVR;VIRPDILPVIK	72	1124;1731	True;True	1219;1904	1545;2366	2256;2257	2257;3311			-1
P62707	P62707	1	1	1	2,3-bisph	gpmA	sp|P6270	1	1	1	1	4.4	4.4	4.4	28.556	250	250	0	8.8065	7126100	1	VIIAAHGNSLR	469	1727	TRUE	1900	2362	3307	3307			-1
P0A6L2	P0A6L2	1	1	1	4-hydroxy	dapA	sp|P0A6L	1	1	1	1	7.2	7.2	7.2	31.27	292	292	0	26.944	7039300	1	IPVIAGTGANATAEAISLTQR	85	844	TRUE	916	1156	1749	1749			-1
P37095	P37095	1	1	1	Peptidase	pepB	sp|P3709	1	1	1	1	2.8	2.8	2.8	46.18	427	427	0.003953	5.6616	6959300	1	EQGYMGLHTVGR	417	426	TRUE	469	577	840	840			-1
P15877	P15877	2	2	2	Quinoprot	gcd	sp|P1587	1	2	2	2	3.8	3.8	3.8	86.746	796	796	0	21.855	6935400	2	GDYVTPTQPFSELSFRPTK;TNESFQHVTCR	335	561;1614	True;True	609;1778	743;2224	1035;3131	1035;3131			-1
P28629	P28629	2	2	2	Biodegrad	adiA	sp|P2862	1	2	2	2	5.8	5.8	5.8	84.424	755	755	0	24.876	6914900	2	LLNALSQASYIHVR;VSILAPGMGEDGELEETG	386	1075;1794	True;True	1165;1978	1466;2462	2136;3445	2136;3445			-1
P37637	P37637	2	2	2	Multidrug	mdtF	sp|P3763	1	2	2	2	4.3	4.3	4.3	111.52	1037	1037	0	13.099	6886800	2	AEAMGVALSDINQTISTAFGSSYVNDFLNQGI	421	41;1686	True;True	43;1856	68;2314	107;3253	107;3253			-1
P0AB77	P0AB77	2	2	2	2-amino-3	kbl	sp|P0AB7	1	2	2	2	6	6	6	43.117	398	398	0	15.218	6881300	2	FICGTQDSHK;TQMSAAHTPEQITR	215	496;1625	True;True	541;1791	660;2240	943;3166	943;3166	173	371	-1
P0AE06	P0AE06	2	2	2	Multidrug	acrA	sp|P0AE0	1	2	2	2	7.8	7.8	7.8	42.196	397	397	0	15.694	6878800	2	AIFPNPDHTLLPGMFVR;IAEVRPQVSGIILK	265	92;736	True;True	101;800	136;999	185;1462	185;1462	210	291	-1
P17952	P17952	2	2	2	UDP-N-ac	murC	sp|P1795	1	2	2	2	9.6	9.6	9.6	53.625	491	491	0	54.769	6797800	3	DASVVVVSSAISADNPEIVAAHEAR;SGTAMI	342	230;1488	True;True	257;1642	315;2067;2	452;2940;2	452;2941			-1
P0AC44	P0AC44	2	2	2	Succinate	sdhD	sp|P0AC4	1	2	2	2	17.4	17.4	17.4	12.867	115	115	0	10.97	6700300	3	MVSNASALGR;NGVHDFILVR	243	1221;1246	True;True	1335;1365	1675;1676	2423;2424	2423;2466			-1
P09831	P09831	3	3	3	Glutamate	gltB	sp|P0983	1	3	3	3	3.2	3.2	3.2	163.3	1486	1486	0	22.044	6667800	3	FGVTPAYLVNADVIQIK;GLITEHVQHTGSQR;	69	489;613;1	True;True	534;663;1	653;807;2	936;1160;3	936;1160;3226			-1
P0A8N3	P0A8N3	4	2	2	Lysine--tR	lysS	sp|P0A8N	1	4	2	2	14.3	9.5	9.5	57.603	505	505	0	60.326	6625300	3	ALRPLPDK;DAGDDEAMFYDEDYVTALEHGLP	155	125;224;2	False;True	137;251;2	172;307;3	228;440;4	228;441;4	120	234	-1
P0A6F9	P0A6F9	1	1	1	10 kDa ch	groS	sp|P0A6F	1	1	1	1	9.3	9.3	9.3	10.387	97	97	0	8.4224	6594600	2	MNIRPLHDR	80	1201	TRUE	1309	1649	2391;2392	2392			-1
P00961	P00961	5	5	5	Glycine--t	glyS	sp|P0096	1	5	5	5	8.4	8.4	8.4	76.812	689	689	0	40.063	6563000	5	AWYQDEGYTVDTIQAVLAR;LTNANVVDDVI	27	209;1140;1	True;True	231;1235;1	284;1562;1	413;2277;2	413;2277;2650;3515;3516			-1
P0A870	P0A870	1	1	1	Transaldo	talB	sp|P0A87	1	1	1	1	4.1	4.1	4.1	35.219	317	317	0	10.751	6549800	1	NIGEILELAGCDR	147	1261	TRUE	1381	1739	2510	2510			-1
P0A7E5	P0A7E5	2	2	2	CTP synth	pyrG	sp|P0A7E	1	2	2	2	3.5	3.5	3.5	60.373	545	545	0	10.784	6441500	2	DGHPLFAGFVK;QMAVEIGR	106	245;1374	True;True	272;1514	336;1916	477;2760	477;2760			-1
P0A7A9	P0A7A9	1	1	1	Inorganic	ppa	sp|P0A7A	1	1	1	1	6.2	6.2	6.2	19.703	176	176	0.001984	5.668	6232100	1	AQIAHFFEHYK	104	154	TRUE	171	210	285	285			-1
P39835	P39835	2	2	2	High-affin	gntT	sp|P3983	1	2	2	2	8.2	8.2	8.2	45.966	438	438	0	16.952	6215300	2	GIDKPIPEGLYSAK;TLLYGTILAIPTVILAGPVYA	435	596;1601	True;True	645;1763	786;2203	1132;3106	1132;3106			-1
P0A6Z3	P0A6Z3	2	2	2	Chaperon	htpG	sp|P0A6Z	1	2	2	2	5.4	5.4	5.4	71.422	624	624	0	21.573	6189600	2	FASTHTDSSAQTVSLEDYVSR;YIFELNPDHVL	97	461;1873	True;True	505;2062	622;2577	894;3598	894;3598			-1
P27434	P27434	2	2	2	Cytoskele	rodZ	sp|P2743	1	2	2	2	10.4	10.4	10.4	36.191	337	337	0	15.378	6167000	2	DIEEDKAPADLASTFLR;MNTEATHDQNEALT	383	258;1202	True;True	286;1310	351;1650	496;2393	496;2393	280	1	-1
P0AD27	P0AD27	1	1	1	Inner mer	yejM	sp|P0AD2	1	1	1	1	3.8	3.8	3.8	67.295	586	586	0	57.714	6094800	1	LLHVSTPASEYSQGQDLFNPQR	252	1071	TRUE	1161	1462	2132	2132			-1
P0A9R7	P0A9R7	2	2	2	Cell divisi	ftsE	sp|P0A9R	1	2	2	2	11.7	11.7	11.7	24.439	222	222	0	12.731	6030700	2	IWFSGHDITR;MLTLSDGHLHGGVGHE	193	888;1196	True;True	964;1303	1209;1642	1812;2383	1812;2383			-1
P0A8M3	P0A8M3	2	2	2	Threonine	thrS	sp|P0A8M	1	2	2	2	5.8	5.8	5.8	74.013	642	642	0	26.921	6006600	2	AEADLAVALEENNIPFEYQLGEGAFYGPK;KVI	153	39;945	True;True	41;1026	66;1275	104;1891	104;1891			-1
P0AES6	P0AES6	3	3	3	DNA gyras	gyrB	sp|P0AES	1	3	3	3	6.3	6.3	6.3	89.949	804	804	0	26.608	5940100	4	DGKEDHFHYEGGIK;FDVHTNAEQNLFEPIVR;	278	246;466;1	True;True	273;510;1	337;627;1	478;900;2	478;900;2381			-1
P60752	P60752	2	2	2	Lipid A ex	msbA	sp|P6075	1	2	2	2	7.7	7.7	7.7	64.46	582	582	0	25.486	5805400	2	DSPILILDEATSALDTESER;NQVALVSQNVHLI	459	298;1280	True;True	331;1403	405;1765	584;2540	584;2540			-1
P0ABK2	P0ABK2	1	1	1	Cytochron	cydB	sp|P0ABK	1	1	1	1	2.1	2.1	2.1	42.453	379	379	0	7.4807	5800100	1	TVGELHLR	231	1646	TRUE	1813	2267	3201	3201			-1
P0AEB2	P0AEB2	1	1	1	D-alanyl-[	dacA	sp|P0AEB	1	1	1	1	3.7	3.7	3.7	44.443	403	403	0	9.1306	5506500	1	ASLGVDKDVYLTIPR	270	169	TRUE	187	227	305	305			-1
P37650	P37650	3	3	3	Cellulose	bcsC	sp|P3765	1	3	3	3	4.3	4.3	4.3	127.72	1157	1157	0	24.791	5482500	5	DSEALGALGQAYSQKGDR;RPISSSLLAFGGQ	422	294;1436;1	True;True	327;1583;1	400;1997;2	579;2854;2	579;2855;2903			-1
P37773	P37773	1	1	1	UDP-N-ac	mpl	sp|P3777	1	1	1	1	4.4	4.4	4.4	49.874	457	457	0	7.8952	5466200	1	HVGVAPADAANALGSFINAR	425	723	TRUE	785	980	1443	1443			-1
P0AG27	P0AG27	1	1	1	Uncharact	yibN	sp|P0AG2	1	1	1	1	9.1	9.1	9.1	15.596	143	143	0.005882	5.6052	5457300	1	LINKEDAVVVDLR	301	1052	TRUE	1140	1440	2105	2105			-1
P0AFL6	P0AFL6	3	3	3	Exopolyph	ppx	sp|P0AFL	1	3	3	3	8	8	8	58.135	513	513	0	18.026	5447900	3	LAHPQLEALLR;LIFMGVEHTQPEK;TASSLAN	297	961;1047;1	True;True	1042;1135	1295;1435	1916;2095	1916;2095;3026			-1
P0ABF1	P0ABF1	1	1	1	Poly(A) pc	pcnB	sp|P0ABF	1	1	1	1	4.1	4.1	4.1	53.87	465	465	0	19.376	5442000	1	EESEAEQAVARPQVTVIPR	225	345	TRUE	378	470	680	680			-1
P76658	P76658	1	1	1	Bifunctior	hldE	sp|P7665	1	1	1	1	3.8	3.8	3.8	51.05	477	477	0	19.111	5416000	1	LDFEEGFEGVDPQPLHER	508	979	TRUE	1061	1317	1941	1941			-1
Q59385	Q59385	2	2	2	Copper-ex	copA	sp|Q5938	1	2	2	2	3.2	3.2	3.2	87.872	834	834	0	13.775	5401400	2	ASAVGSHTTLSR;LAAALEQGSSHPLAR	521	164;950	True;True	182;1031	222;1280	299;1897	299;1897			-1
P75796	P75796	2	2	2	Glutathior	gsiA	sp|P7579	1	2	2	2	3.5	3.5	3.5	69.113	623	623	0	12.295	5361100	2	AVFENPQHPYTR;VGLLPEHAWR	490	189;1706	True;True	210;1878	253;2339	336;3279	336;3279			-1
P0A821	P0A821	3	3	3	L-seryl-tR	selA	sp|P0A82	1	3	3	3	8.9	8.9	8.9	50.607	463	463	0	28.436	5307700	3	EPMPQELIAAGVSLVSFSGDK;LPSAALTFTPH	140	423;1103;1	True;True	465;1197;1	573;1520;2	836;2221;3	836;2221;3478			-1
P0AFI7	P0AFI7	1	1	1	Pyridoxine	pdxH	sp|P0AFI	1	1	1	1	6.9	6.9	6.9	25.545	218	218	0	6.3176	5295700	1	VSLEQIEFWQGGEHR	294	1795	TRUE	1979	2463	3446	3446			-1
P43329	P43329	2	2	2	ATP-depe	hrpA	sp|P4332	1	2	2	2	2.2	2.2	2.2	149.03	1300	1300	0	13.54	5295600	2	HFNNAPIIEVSGR;LLMQYDTIIIDEAHER	437	680;1074	True;True	737;1164	921;1465	1327;2135	1327;2135			-1
P32169	P32169	1	1	1	Rhamnulc	rhaD	sp|P3216	1	1	1	1	4.4	4.4	4.4	30.145	274	274	0	6.0733	5265300	1	FGVTPLASALAL	401	490	TRUE	535	654	937	937			-1
P77488	P77488	2	2	2	1-deoxy-[	dxs	sp|P7748	1	2	2	2	4.8	4.8	4.8	67.616	620	620	0	11.876	5224600	2	AYDQVLHDVAIQK;QMLYTGYHYNDGPSAVR	514	210;1375	True;True	232;1515	285;1917	414;2761	414;2761			-1
P04079	P04079	3	3	3	GMP synth	guaA	sp|P0407	1	3	3	3	5.9	5.9	5.9	58.679	525	525	0	33.109	5211300	3	FLSALAGENDPEAK;RADAIFIEELR;VLGEVK	36	511;1404;1	True;True	556;1548;1	681;1956;2	969;2805;3	969;2805;3334			-1
P0ACL9	P0ACL9	2	2	2	Pyruvate	pdhR	sp|P0ACL	1	2	2	2	11	11	11	29.425	254	254	0	11.396	5100900	2	HALEGIAAYYAALR;IFEAIMAGKPEEAR	246	673;773	True;True	727;838	902;1056	1288;1585	1288;1585			-1
P0AA43	P0AA43	1	1	1	Ribosoma	rsuA	sp|P0AA4	1	1	1	1	9.1	9.1	9.1	25.865	231	231	0	15.843	5079400	3	LLPEHDVAYDGNPLAQQHGPR	201	1076	TRUE	1166	1467;1468	2137;2138	2137			-1
P21165	P21165	2	2	2	Xaa-Pro di	pepQ	sp|P2116	1	2	2	2	4.7	4.7	4.7	50.176	443	443	0	11.63	5061200	3	GVIDYLHYYR;IEALKPFGGIR	347	656;757	True;True	710;822	883;1040	1265;1567	1265;1567			-1
P0AAT6	P0AAT6	1	1	1	Ribosoma	rsfS	sp|P0AAT	1	1	1	1	13.3	13.3	13.3	11.582	105	105	0	12.144	5043300	1	HVMSIADHVVQESR	209	724	TRUE	786	981	1444	1444			-1
P08506	P08506	1	1	1	D-alanyl-[	dacC	sp|P0850	1	1	1	1	4.5	4.5	4.5	43.608	400	400	0	32.795	5043100	2	FFETVTPIKPDATFVTQR	61	473	TRUE	517	634	908;909	908			-1
P0A993	P0A993	2	2	2	Fructose-1	fbp	sp|P0A99	1	2	2	2	6.9	6.9	6.9	36.833	332	332	0	15.405	5039300	2	ILDIIPETLHQR;YIGSLVADFHR	176	816;1874	True;True	884;2063	1114;2578	1668;3599	1668;3599			-1
P76576	P76576	1	1	1	UPF0070 p	yfgM	sp|P7657	1	1	1	1	12.6	12.6	12.6	22.176	206	206	0	39.106	5009500	1	SASLAYQNAVTAVSEGKPDSIPAAEK	506	1455	TRUE	1607	2027	2897	2897			-1
P41052	P41052	1	1	1	Membran	mltB	sp|P4105	1	1	1	1	7.5	7.5	7.5	40.255	361	361	0	31.002	4991800	1	YSISQLAAAGLTPQQPLGNHQQASLLR	436	1907	TRUE	2100	2628	3723	3723			-1
P06993	P06993	3	3	3	HTH-type	malT	sp|P0699	1	3	3	3	5.5	5.5	5.5	103.12	901	901	0	30.229	4986000	3	AQILLGEFEPAEIVLEELNENAR;EDTMHAEFN	47	155;338;1	True;True	172;371;1	211;463;1	286;672;2	286;672;2737			-1
P0AF98	P0AF98	1	1	1	Lipopolys	lptF	sp|P0AF9	1	1	1	1	4.1	4.1	4.1	40.357	366	366	0	31.473	4979000	1	PSVVVADSGHLTQLR	281	1317	TRUE	1450	1832	2646	2646			-1
P0A921	P0A921	1	1	1	Phospholi	pldA	sp|P0A92	1	1	1	1	4.5	4.5	4.5	33.163	289	289	0	5.8485	4978800	1	IGYHLGDAVLSAK	168	787	TRUE	852	1072	1602	1602			-1
P68767	P68767	2	2	2	Cytosol ar	pepA	sp|P6876	1	2	2	2	8.5	8.5	8.5	54.879	503	503	0	44.532	4943700	2	AYRPGDVLTTMSGQTVEVLNTDAEGR;GATG	477	213;550	True;True	236;598	290;730	421;1022	421;1022	314	341	-1
P25553	P25553	1	1	1	Lactaldeh	aldA	sp|P2555	1	1	1	1	4	4	4	52.272	479	479	0	8.0294	4913500	1	YEGEIIQSDRPGENILLFK	373	1864	TRUE	2052	2563	3574	3574			-1
P0ADR8	P0ADR8	1	1	1	LOG famil	ygdH	sp|P0ADR	1	1	1	1	2.9	2.9	2.9	50.971	454	454	0	16.876	4791800	1	MDDLLQGFVAQHR	259	1171	TRUE	1271	1607	2339	2339			-1
P08395	P08395	1	1	1	Protease	sppA	sp|P0839	1	1	1	1	3.2	3.2	3.2	67.219	618	618	0	10.858	4739200	1	GALLLDISGVIVDKPDSSQR	60	546	TRUE	593	724	1016	1016			-1



C. Total Ion Chromatogram (TIC) of a) BS3 and b) DSBU-cross-linked solubilized membrane samples.



D. Total number of interactions.

Interaction Type	**Number of Interaction**	
Complex Inter-Protein	30	
Complex Intra-Protein	119	
Complex-Other	218	
Other Proteins Inter-Protein	372	
Other Proteins Intra-Protein	354	
MaxLynx	**Purified Protein Complex**	**Solubilized Membrane**
Complex Inter-Protein	11	4
Complex Intra-Protein	33	23
Complex-Protein-Protein	8	1
Other Protein Interaction	2	100
MeroX	**Purified Protein Complex**	**Solubilized Membrane**
Complex Inter-Protein	8	6
Complex Intra-Protein	50	43
Complex-Protein-Protein	10	1
Other Protein Interaction	5	204
MS Annika	**Purified Protein Complex**	**Solubilized Membrane**
Complex Inter-Protein	10	2
Complex Intra-Protein	37	7
Complex-Protein-Protein	146	3
Other Protein Interaction	300	0
XlinkX	**Purified Protein Complex**	**Solubilized Membrane**
Complex Inter-Protein	8	4
Complex Intra-Protein	39	21
Complex-Protein-Protein	45	10
Other Protein Interaction	7	184

E. Table of intra-protein interactions of three proteins in the complex obtained in two samples (purified protein, solubilized membrane) analyzed through four software, MaxLynx, MeroX, MS Annika, XlinkX.

			MaxLynx		MeroX		MS Annika		XlinkX	
		Connection Points	Purified System	Solubilized Membrane	Purified System	Solubilized Membrane	Purified System	Solubilized Membrane	Purified System	Solubilized Membrane
Intra-protein Interactions	FtsH	494-550	+		+*		+		+	+
		31-60	+		+*		+			
		31-31	+		+					
		494-533	+	+	+*	+*	+		+	
		148-203	+	+	+*	+*	+		+	+
		387-332	+		+				+	
		433-387	+	+	+*		+		+	
		580-332	+	+	+*	+*	+			
		203-332	+		+*		+		+	
		535-533	+	+	+	+	+		+	+
		203-61	+							
		550-535	+		+	+	+		+	
		332-332	+		+					
		550-179	+				+			
		242-203	+				+			
		408-332	+				+		+	
		620-332	+						+	+
		203-179	+			+			+	+
		550-61	+							
		408-387	+				+		+	
		461-387	+						+	
		433-61	+							
		433-203	+							
		76-391	+				+			
		179-179	+	+		+	+		+	
		550-550			+					
		387-391		+	+	+	+		+	+
		533-533		+	+	+				
		461-550			+					
		31-179			+		+			
		332-391			+					
		387-550			+				+	
		332-461/464/467/468/470			+*					
		456/458-461			+*					
		494-535			+*		+		+	+
		535-535			+*	+				
		332-433/435			+*					
		391-433			+*	+*	+		+	+
		408-580			+*	+*	+		+	
		410/411/413-580				+*				
		332-76					+			
		533-550				+	+			
		332-148					+			
		550-433					+			
		179-332				+	+		+	
		391-494					+			
		179-76					+			
		332-550					+		+	
		84-76				+*	+			
		203-494					+			
		433-183							+	
		203-183		+		+			+	+
		186-179							+	
		408-391							+	
		183-550							+	
		433-179							+	
		494-183							+	
		494-387							+	
		183-332							+	
		408-389							+	
		76-76				+				
		494-541				+*				
		186-203				+				+
		179-387				+				
		179-391				+				+
		136/137-179				+*				
		433/435-461/464				+*				
		203-136				+*				+
		183-391								+
		461-533								+
	HflC	63-36	+	+	+*	+	+	+	+	+
		137-85	+		+*	+*	+	+	+	+
		120-211	+						+	
		44-36	+		+	+	+	+		+
		327-327	+	+	+*	+*	+	+		
		187-36	+	+		+	+	+	+	+
		327-328/330/331/333			+*					
		137-186			+					
		248-327			+				+	
		327-333			+					
		82-85		+	+*	+*				
		82-137		+	+*		+	+	+	+
		187-44		+			+	+		+
		84-137		+					+	+
	HflK	294-310	+	+	+*	+*	+		+	
		368-368	+	+		+				

*Unspecific connections

F. The most abundant 10 inter- and intra-protein interactions from solubilized membrane and purified protein samples.

		MaxLynx											
		Peptide 1	Peptide 2	Protein 1	Protein 2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Purified System Intraprotein Interactions	FtsH	LAEEIIYGPEHVSTGASNDIKVATNLAR	IIDQEVKALIER	FtsH	FtsH	494	550	271.46	1	4	1148.3658	4590.44193	3
		KVDYSTFLQEVNNDQVR	EINVTKK	FtsH	FtsH	31	60	205.39	2	3	1027.8666	3081.58563	1
		KVDYSTFLQEVNNDQVR	KVDYSTFLQEVNNDQVR	FtsH	FtsH	31	31	180.42	3	3	1435.7068	4305.10643	3
		VVSMVEFEKAK	EQILKVHMR	FtsH	FtsH	387	332	118.97	10	3	872.47311	2615.40533	1
		LAEEIIYGPEHVSTGASNDIKVATNLAR	SVAKAK	FtsH	FtsH	494	533	139.69	7	3	1256.3373	3766.99793	2
		[M]LTEDQIKTTFADVAGCDEAK	TLLAKAIAGEAK	FtsH	FtsH	148	203	127.46	8	3	1247.2971	3739.87743	3
	HflC	IPFIETVKMLDAR	FGKVLR	HflC	HflC	63	36	171.75	4	3	816.46628	2447.38483	2
		LDVKDIVTDSR	KDLIVDSYIK	HflC	HflC	137	85	148.28	5	4	663.11409	2649.43513	1
		YYLATGGGDISQAEVLLKR	IKQINLPTEVSEAIYNR	HflC	HflC	120	211	141.71	6	4	1060.068	4237.25083	2
		DDDNKPLVYEPGLHFK	FGKVLR	HflC	HflC	44	36	120.99	9	3	934.49221	2801.46263	1
	HflK												

		MaxLynx											
		Peptide 1	Peptide 2	Protein 1	Protein 2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Purified System Interprotein Interactions	FtsH and HflK	KVDYSTFLQEVNNDQVR	PTFIDEVKPVNVEAVR	FtsH	HflK	31	133	134.93	1	4	1016.5247	4063.07763	2
	FtsH and HflC	YYLATGGGDISQAEVLLKR	LLDNLLTKNVK	HflC	FtsH	120	84	88.564	3	3	1173.9856	3519.94263	1
		KVDYSTFLQEVNNDQVR	IPFIETVK[M]LDAR	FtsH	HflC	31	63	83.41	5	4	950.48973	3798.93763	6
		IKQINLPTEVSEAIYNR	KDSNR	HflC	FtsH	211	61	56.079	8	3	934.82967	2802.47503	1
	HflK and HflC	VTNPEKYLYSVTSPDDSLR	FGKVLR	HflK	HflC	172	36	89.159	2	3	1033.5434	3098.61623	1
		LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	137	198	86.543	4	3	865.78641	2595.34523	3
		VLVNDKGGNLMVLPLDQ[M]LK	YMKTPTSATR	HflK	HflC	346	327	83.375	6	3	1188.626	3563.86393	6
		VTNPEKYLYSVTSPDDSLR	KFSDR	HflK	HflC	172	122	68.741	7	3	1011.1718	3031.50123	1
		AYKAQTILEAQGEVAR	Y[M]KTPTSATR	HflK	HflC	294	327	54.745	9	4	779.40266	3114.58933	2
		GEGDAEAAKLFADAFSK	AYKAQTILEAQGEVAR	HflC	HflK	281	294	52.443	10	3	1223.9513	3669.84003	1

		MaxLynx											
		Peptide1	Peptide2	Protein1	Protein2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Cell Lysate Intraprotein Interactions	FtsH	VVSMVEFEKAK	DKIMMGAER	FtsH	FtsH	387	391	198.68	2	3	838.09179	2512.26133	10
		LAEEIIYGPEHVSTGASNDIKVATNLAR	SVAKAK	FtsH	FtsH	494	533	145.6	7	3	1256.3373	3766.99793	2
		TLLAKAIAGEAK	LGGKIPK	FtsH	FtsH	203	183	125.97	8	3	698.42793	2093.26983	2
		IIDQEVKALIER	SVAKAK	FtsH	FtsH	550	533	119.26	9	4	557.07705	2225.28693	1
		MLTEDQIKTTFADVAGCDEAK	TLLAKAIAGEAK	FtsH	FtsH	148	203	153.82	5	3	1241.9655	3723.88253	2
	HflC	EKKDLIVDSYIK*	LDVKDIVTDSR	HflC	HflC	84	137	190.28	3	4	727.39848	2906.57263	3
		VTAETKGK	FGKVLR	HflC	HflC	187	36	149.35	6	3	583.34035	1748.00703	1
		LDVKDIVTDSR	FVTKEK	HflC	HflC	137	82	118.19	10	3	736.40211	2207.19233	1
	HflK	AYKAQTILEAQGEVAR	FAKLLPEYK	HflK	HflK	294	310	211.89	1	3	1017.8886	3051.65193	1
		GGNAPAAKSDNGASNLLR	GGNAPAAKSDNGASNLLR	HflK	HflK	368	368	157.43	4	4	905.95855	3620.81293	1

		MaxLynx											
		Peptide1	Peptide2	Protein1	Protein2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Cell Lysate Interprotein Interactions	FtsH and HflK												
	FtsH and HflC	YYLATGGGDISQAEVLLKR	KDSNR	HflC	FtsH	120	61	80.205	4	3	956.83319	2868.48553	1
	HflK and HflC	AYKAQTILEAQGEVAR	SQGQEEAEKLR	HflK	HflC	294	248	201.62	1	3	1073.2198	3217.64533	3
		LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	137	198	143.27	2	3	865.78641	2595.34523	4
		VLVNDKGGNLMVLPLDQMLK	Y[M]KTPTSATR	HflK	HflC	346	327	106.85	3	4	891.72131	3563.86393	1

[] Modified Amino Acids
* Extra miscleavage

Not verified

		MeroX											
		Peptide 1	Peptide 2	Protein 1	Protein 2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Purified System Intraprotein Interactions	FtsH	LAEEIIYGPEHVSTGASNDIKVATNLAR	IIDQEVKALIER	FtsH	FtsH	494/497	550	150	9	4	1148.37146	4590.464011	4
		KVDYSTFLQEVNNDQVR	KVDYSTFLQEVNNDQVR	FtsH	FtsH	31	31	190	4	4	1077.032593	4305.108542	1
		[M]LTEDQIKTTFADVAGBDEAK	TLLAKAIAGEAK	FtsH	FtsH	148/149/150	203	200	3	4	935.7270508	3739.886374	1
		TLLAKAIAGEAK	EQILKVH[M]R	FtsH	FtsH	203	332	143	10	4	638.368042	2550.450339	2
		IIDQEVKALIER	IIDQEVKALIER	FtsH	FtsH	550	550	217	1	4	762.9437866	3048.753317	1
		VVSMVEFEKAK	DKIMMGAER	FtsH	FtsH	387	391	202	2	3	838.0932617	2512.265232	4
	HflC	FGKVLR	IPFIETVKMLDAR	HflC	HflC	36	63	179	5	4	612.6036377	2447.392722	4
		KDLIVDSYIK	LDVKDIVTDSR	HflC	HflC	85	137	178	6	4	663.1153564	2649.439597	2
		Y[M]KTPTSATR	Y[M]KTPTSATR	HflC	HflC	327	327	176	7	4	635.3143921	2538.235739	4
	HflK	AYKAQTILEAQGEVAR	FAKLLPEYK	HflK	HflK	293/294	310	173	8	3	1017.890869	3051.658055	3

		MeroX											
		Peptide 1	Peptide 2	Protein 1	Protein 2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Purified System Interprotein Interactions	FtsH and HflK												
	FtsH and HflC	KVDYSTFLQEVNNDQVR	IPFIETVKMLDAR	FtsH	HflC	31	63	127	4	4	946.4923096	3782.947409	1
		KVDYSTFLQEVNNDQVR	IPFIETVK[M]LDAR	FtsH	HflC	31	61/63	89	6	4	950.4899902	3798.938132	2
	HflK and HflC	LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	137	198	190	1	4	649.5930786	2595.350485	1
		SEIGRLDVKDIVTDSR*	GVIGKYTMDR	HflC	HflK	137	198/199/200	172	2	5	628.3331299	3137.636544	10
		LDVKDIVTDSR	GVIGKYT[M]DR	HflC	HflK	141/143	198	131	3	4	653.5912476	2611.343161	5
		LDVKDIVTDSR	GVIGKYT[M]DR	HflC	HflK	137/141	198/199/200	42	9	3	871.1190796	2611.342686	2
		LDVKDIVTDSR	GVIGKYT[M]DR	HflC	HflK	137/141/143	198/199/200	80	7	3	871.1191406	2611.342869	3
		AYKAQTILEAQGEVAR	YMKTPTSATR	HflK	HflC	294	328/330/331/333	90	5	4	775.4056396	3098.600729	1
		AYKAQTILEAQGEVAR	Y[M]KTPTSATR	HflK	HflC	293/294/297	327/328/330/331/333	42	10	4	779.4036865	3114.592917	1
		SQGQEEAEKLR	AYKAQTILEAQGEVAR	HflC	HflK	248	293/294	77	8	4	805.1678467	3217.649558	1

		MeroX											
		Peptide1	Peptide2	Protein1	Protein2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Cell Lysate Intraprotein Interactions	FtsH	VVSMVEFEKAK	DKIMMGAER	FtsH	FtsH	387	391	212	4	4	628.8223267	2512.267478	4
		LGGKIPK	TLLAKAIAGEAK	FtsH	FtsH	183	203	153	10	4	524.072937	2093.269919	2
		MLTEDQIKTTFADVAGBDEAK	TLLAKAIAGEAK	FtsH	FtsH	148/149/150	203	183	6	4	931.7333984	3723.911765	1
		MLTEDQIKTTFADVAGBDEAK	TLLAKAIAGEAK	FtsH	FtsH	148	203	166	7	4	931.7238159	3723.873435	2
		AKHMSDETAR	AKHMSDETAR	FtsH	FtsH	535	535	236	2	4	622.2925415	2486.148337	2
		YTTYIPVQDPKLLDNLLTK	YTTYIPVQDPKLLDNLLTK	FtsH	FtsH	76	76	163	8	4	1167.139648	4665.536765	1
	HflC	KDLIVDSYIK	LDVKDIVTDSR	HflC	HflC	85	137	187	5	4	663.1148071	2649.437399	4
	HflK	AYKAQTILEAQGEVAR	FAKLLPEYK	HflK	HflK	294	310	222	3	3	1017.890381	3051.65659	1
		AYKAQTILEAQGEVAR	FAKLLPEYK	HflK	HflK	293/294	310	158	9	4	763.6702881	3051.659323	3
		GGNAPAAKSDNGASNLLR	GGNAPAAKSDNGASNLLR	HflK	HflK	368	368	258	1	4	905.9602661	3620.819235	1

		MeroX											
		Peptide1	Peptide2	Protein1	Protein2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Cell Lysate Interprotein Interactions	FtsH and HflK												
	FtsH and HflC	KVDYSTFLQEVNNDQVR	IPFIETVKMLDAR	FtsH	HflC	31/34/35/36	63	10	10	4	946.4918213	3782.945456	1
	HflK and HflC	SQGQEEAEKLR	AYKAQTILEAQGEVAR	HflC	HflK	248	293/294	185	2	4	805.166687	3217.644919	1
		SQGQEEAEKLR	AYKAQTILEAQGEVAR	HflC	HflK	248	294	84	7	3	1073.217163	3217.636936	1
		LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	137	198	170	3	4	649.5924072	2595.3478	2
		LDVKDIVTDSR	GVIGKYT[M]DR	HflC	HflK	137	198/199/200	101	6	3	871.1171265	2611.336827	1
		YMKTPTSATR	VLVNDKGGNLMVLPLDQMLK	HflC	HflK	327	346	139	5	4	887.7294312	3547.895895	2
		YMKTPTSATR	VLVNDKGGNLMVLPLDQMLK	HflC	HflK	331/333	346	13	9	3	1183.304199	3547.898045	1
		GEGDAEAAKLFADAFSK	FAKLLPEYK	HflC	HflK	281	310	202	1	4	758.3927612	3030.549216	1
		LFADAFSKDPDFYAFIR	FAKLLPEYK	HflC	HflK	288/289/294	310	60	8	4	832.4420166	3326.746237	1
		LDVKDIVTDSR	GVIGKYT[M]DR	HflC	HflK	141	198	143	4	4	653.5905151	2611.340231	1

[] Modified Amino Acids
* Extra miscleavage

		MS Annika											
		Peptide 1	Peptide 2	Protein 1	Protein 2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Purified System Intraprotein Interactions	FtsH	IIDQEVKALIER	EEIIYGPEHVSTGASNDIKVATNL	FtsH	FtsH	550	494	342.92	4	5	918.895752	4590.449654	4
		SVAKAK	EEIIYGPEHVSTGASNDIKVATNL	FtsH	FtsH	533	494	225.4	10	3	1256.344238	3767.018162	13
		TLLAKAIAGEAK	MLTEDQIKTTFADVAG[C]DEAK	FtsH	FtsH	203	148	538.37	1	3	1241.967896	3723.889134	3
													S[M
													F
													S
		DALMKYETIDAPQIDDL[M]AR	MVMTEAQKESTAYHEAGHAIIG	FtsH	FtsH	580	408	434.57	2	4	1285.122314	5137.467428	4
		EQILKVH[M]R	TLLAKAIAGEAK	FtsH	FtsH	332	203	265.73	7	4	638.368286	2550.451315	1
		VVSMVEFEKAK	LVPEHDPVHKVTIIPR	FtsH	FtsH	387	433	267.19	6	3	1104.614624	3311.829319	1
		AKHMSDETAR	IIDQEVKALIER	FtsH	FtsH	535	550	229.4	9	3	923.15387	2767.447056	3
	HflC	KDLIVDSYIK	LDVKDIVTDSR	HflC	HflC	85	137	394.28	3	4	663.114929	2649.437887	2
		LDVKDIVTDSR	EKKDLIVDSYIK	HflC	HflC	137	85	300.01	5	3	969.53064	2906.577366	1
	HflK	FAKLLPEYK	AYKAQTILEAQGEVAR	HflK	HflK	310	294	244.89	8	4	763.669434	3051.655905	1

		MS Annika											
		Peptide 1	Peptide 2	Protein 1	Protein 2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Purified System Interprotein Interactions	FtsH and HflK												
	FtsH and HflC	LLDNLLTKNVK	YYLATGGGDISQAEVLLKR	FtsH	HflC	84	120	171.42	6	3	1173.987183	3519.946995	1
		IPFIETVK[M]LDAR	KVDYSTFLQEVNNDQVR	HflC	FtsH	63	31	420.74	1	3	1266.989258	3798.953221	3
		KDSNR	IKQINLPTEVSEAIYNR	FtsH	HflC	61	211	70.07	10	3	934.83313	2802.484837	1
		EINVTKK	YYLATGGGDISQAEVLLKR	FtsH	HflC	60	120	178.64	5	4	770.922302	3080.66738	1
		EINVTKK	IKQINLPTEVSEAIYNR	FtsH	HflC	60	211	118.4	7	3	1005.558044	3014.65958	2
		KDSNR	YYLATGGGDISQAEVLLKR	FtsH	HflC	61	120	78.99	9	4	717.879578	2868.496481	4
	HflK and HflC	GVIGKYTMDR	LDVKDIVTDSR	HflK	HflC	198	137	387.4	2	4	649.594055	2595.354391	15
		Y[M]KTPTSATR	VLVNDKGGNLMVLPLDQMLK	HflC	HflK	327	346	319.82	3	4	891.7229	3563.869772	7
		KFSDR	VTNPEKYLYSVTSPDDSLR	HflC	HflK	122	172	102.68	8	3	1011.173218	3031.5051	1
		SQGQEEAEKLR	AYKAQTILEAQGEVAR	HflC	HflK	248	294	247.61	4	3	1073.557861	3218.659031	1

		MS Annika											
		Peptide1	Peptide2	Protein1	Protein2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Cell Lysate Intraprotein Interactions	FtsH	DKIMMGAER	VVSMVEFEKAK	FtsH	FtsH	391	387	224.26	9	3	838.093567	2512.266148	5
		SVAKAK	EEIIYGPEHVSTGASNDIKVATNL	FtsH	FtsH	533	494	255.69	8	3	1256.338135	3766.999851	16
		TLLAKAIAGEAK	MLTEDQIKTTFADVAG[C]DEAK	FtsH	FtsH	203	148	653.17	1	3	1241.966919	3723.886204	3
		TLLAKAIAGEAK	IPKGVLMVGPPGTGK	FtsH	FtsH	203	186	332.91	4	4	708.670471	2831.660055	2
		DALMKYETIDAPQIDDL[M]AR	MVMTEAQKESTAYHEAGHAIIG	FtsH	FtsH	580	408	272.04	5	5	1028.496948	5138.455635	1
		EQILKVHMR	DALMKYETIDAPQIDDLMAR	FtsH	FtsH	332	580	258.05	7	3	1220.292847	3658.863987	5
		GAMSFGKSK	TLLAKAIAGEAK	FtsH	FtsH	136	203	216.36	10	3	765.094666	2293.269444	1
													LAE
													DVRPPAG
	HflC	KDLIVDSYIK	LDVKDIVTDSR	HflC	HflC	85	137	401.07	2	3	883.822632	2649.453343	2
		LDVKDIVTDSR	EKKDLIVDSYIK*	HflC	HflC	137	85	355.12	3	3	969.527893	2906.569126	2
	HflK	FAKLLPEYK	AYKAQTILEAQGEVAR	HflK	HflK	310	294	265.46	6	5	611.140137	3051.671578	8

		MS Annika											
		Peptide1	Peptide2	Protein1	Protein2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
Cell Lysate Interprotein Interactions	FtsH and HflK												S
	FtsH and HflC	KDSNR	YYLATGGGDISQAEVLLKR	FtsH	HflC	61	120	79.36	4	4	717.875977	2868.482077	4
	HflK and HflC	GVIGKYTMDR	LDVKDIVTDSR	HflK	HflC	198	137	342.75	2	3	865.787781	2595.348789	3
		Y[M]KTPTSATR	VLVNDKGGNLMVLPLDQMLK	HflC	HflK	327	346	195.31	3	4	891.723328	3563.871481	1
		FAKLLPEYK	GEGDAEAAKLFADAFSK	HflK	HflC	310	281	352.56	1	4	758.391174	3030.542868	3

[] Modified Amino Acids
* Extra miscleavage

Purified System Intraprotein Interactions

		XlinkX											
		Peptide 1	Peptide 2	Protein 1	Protein 2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
	FtsH	LAEEIIYGPEHVSTGASNDIKVATNLAR	IIDQEVKALIER	FtsH	FtsH	494	550	163.84	2	4	1148.370361	4590.459616	3
		[M]LTEDQIKTTFADVAG[C]DEAK	TLLAKAIAGEAK	FtsH	FtsH	148	203	158.38	3	3	1247.304443	3739.898777	1
		S[M]V[M]TEAQKESTAYHEAGHAIIGR	VVSMVEFEKAKDKIMMGAER	FtsH	FtsH	408	391	125.57	6	5	1068.520874	5338.575264	1
		FQKLGGKIPKGVLMVGPPGTGK	IIDQEVKALIER	FtsH	FtsH	183	550	116.23	8	4	1007.577209	4027.287008	4
		SMVMTEAQKESTAYHEAGHAIIGR	EQILKVHMR	FtsH	FtsH	408	332	106.84	9	4	992.252807	3965.989401	2
		S[M]V[M]TEAQKESTAYHEAGHAIIGR	DAL[M]KYETIDAPQIDDLMAR	FtsH	FtsH	408	580	116.55	7	4	1293.115478	5169.440084	1
	HflC	FVTKEKKDLIVDSYIK*	SEIGRLDVKDIVTDSR*	HflC	HflC	85	137	215.31	1	4	895.243652	3577.95278	21
		YYLATGGGDISQAEVLLKR	IKQINLPTEVSEAIYNR	HflC	HflC	120	211	155.58	4	4	1060.052734	4237.189108	1
		FVTKEKKDLIVDSYIK*	LDVKDIVTDSR	HflC	HflC	84	137	131.3	5	3	969.53064	2906.577366	5
		FVTKEKKDLIVDSYIK*	SEIGRLDVKDIVTDSR*	HflC	HflC	82	137	100.02	10	4	895.242187	3577.94692	14
	HflK												

Purified System Interprotein Interactions

		XlinkX											
		Peptide 1	Peptide 2	Protein 1	Protein 2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
	FtsH and HflK												
	FtsH and HflC	YYLATGGGDISQAEVLLKR	LLDNLLTKNVK	HflC	FtsH	120	84	62.72	6	3	1173.987182	3519.946995	1
		KVDYSTFLQEVNNDQVR	IPFIETVK[M]LDAR	FtsH	HflC	31	63	136.35	2	3	1266.989258	3798.95322	1
		IKQINLPTEVSEAIYNR	KDSNR	HflC	FtsH	211	61	32.32	10	3	934.83313	2802.484837	1
	HflK and HflC	SEIGRLDVKDIVTDSR*	GVIGKYT[M]DR	HflC	HflK	137	198	111.69	3	4	653.598022	2611.37026	22
		VLVNDKGGNLMVLPLDQMLK	Y[M]KTPTSATR	HflK	HflC	346	327	55.44	7	3	1188.631592	3563.880222	2
		AYKAQTILEAQGEVAR	GEGDAEAAKLFADAFSK	HflK	HflC	294	281	65.35	5	3	1223.956665	3669.855442	1
		AYKAQTILEAQGEVAR	SQGQEEAEKLR	HflK	HflC	294	248	142.11	1	3	1073.230591	3217.677219	3
		FVTKEKKDLIVDSYIK*	GVIGKYTMDR	HflC	HflK	82	198	66.27	4	4	864.968872	3456.853659	1
		FVTKEKKDLIVDSYIK*	GVIGKYTMDR	HflC	HflK	84	198	50.02	8	3	1152.956055	3456.853611	1
		FVTKEKKDLIVDSYIK*	GVIGKYT[M]DR	HflC	HflK	85	198	44.14	9	4	868.971741	3472.865133	1

Cell Lysate Intraprotein Interactions

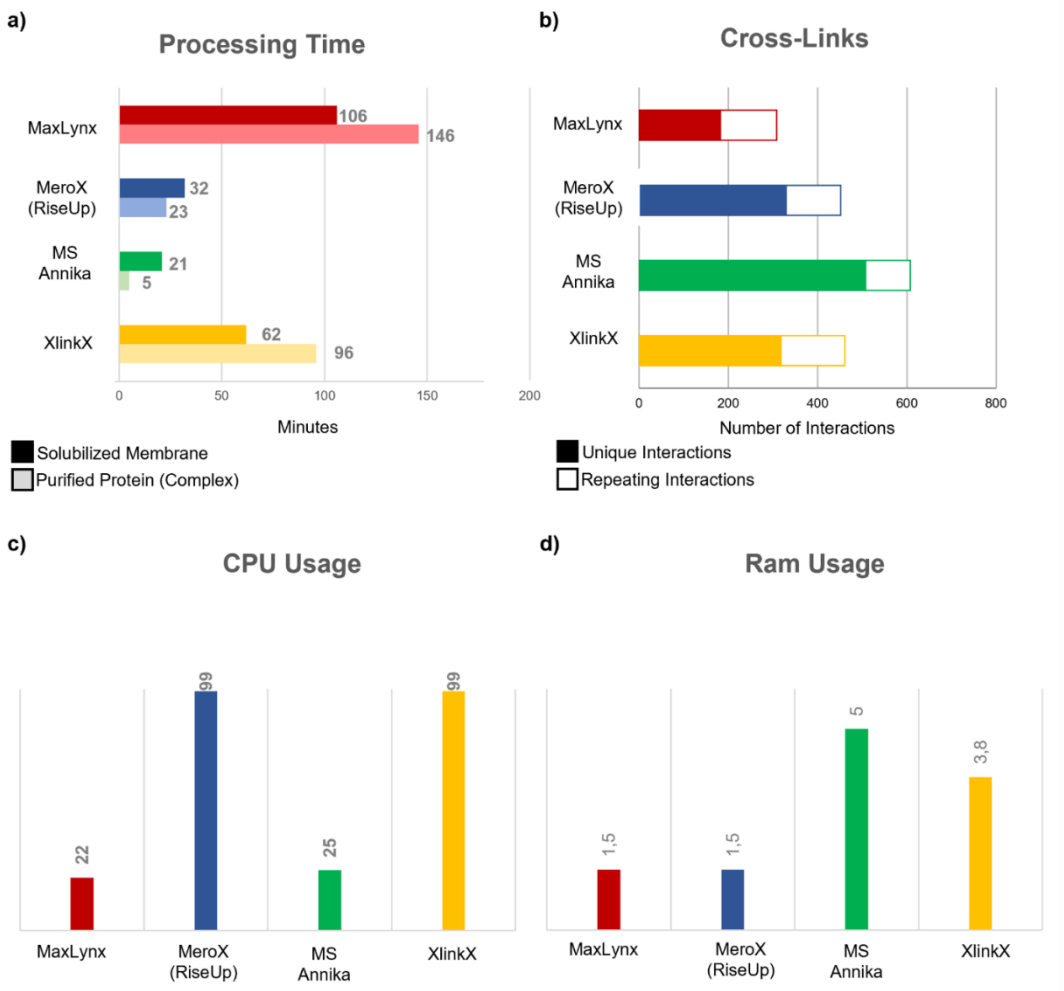
		XlinkX											
		Peptide1	Peptide2	Protein1	Protein2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
	FtsH	[M]LTEDQIKTTFADVAG[C]DEAK	TLLAKAIAGEAK	FtsH	FtsH	148	203	121.29	5	4	935.735657	3739.920797	1
		LAEEIIYGPEHVSTGASNDIKVATNLAR	IIDQEVKALIER	FtsH	FtsH	494	550	148.21	4	4	1148.375976	4590.482077	1
		TLLAKAIAGEAK	FQKLGGKIPK	FtsH	FtsH	203	183	92.69	6	3	904.200317	2710.586399	3
		IPKGVLMVGPPGTGK	TLLAKAIAGEAK	FtsH	FtsH	186	203	82.28	7	4	708.670471	2831.660055	2
		PAGWEEPGASNNSGDNGSPKAPRPV	EQILKVHMR	FtsH	FtsH	620	332	80.38	8	5	961.676575	4804.353767	1
		SVAKAKHMSDETAR*	SVAKAK	FtsH	FtsH	535	533	77.93	9	4	636.58728	2543.327291	5
		DKIMMGAER	FQKLGGK	FtsH	FtsH	391	179	72.05	10	3	675.020996	2023.048435	2
	HflC	FVTKEKKDLIVDSYIK*	LDVKDIVTDSR	HflC	HflC	84	137	215.31	1	4	895.240661	3577.940817	4
		FVTKEKKDLIVDSYIK*	LDVKDIVTDSR	HflC	HflC	82	137	180.93	2	4	895.239929	3577.937887	2
		FVTKEKKDLIVDSYIK*	LDVKDIVTDSR	HflC	HflC	85	137	175.94	3	4	895.249328	3577.975485	8
	HflK												

Cell Lysate Interprotein Interactions

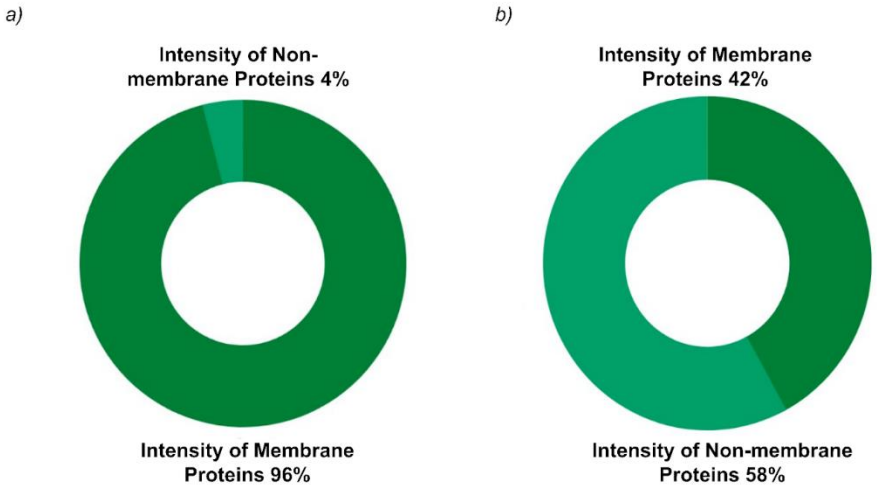
		XlinkX											
		Peptide1	Peptide2	Protein1	Protein2	From	To	Score	Score Rank	Charge	m/z	M+H+	Number of spectra matching to this site
	FtsH and HflK	SMVMTEAQKESTAYHEAGHAIIGR	SGNLMVLPLDQMLKGGNAPAAI	FtsH	HflK	408	360	12.38	5	6	835.421631	5007.493402	1
	FtsH and HflC												
	HflK and HflC	AYKAQTILEAQGEVAR	SQGQEEAEKLR	HflK	HflC	294	248	126.24	2	3	1073.217163	3217.636936	1
		LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	137	198	117.56	3	3	865.787781	2595.348789	4
		GEGDAEAAKLFADAFSK	FAKLLPEYK	HflC	HflK	281	310	62.84	4	3	1010.864441	3030.57877	1
		AYKAQTILEAQGEVAR	GEGDAEAAKLFADAFSK	HflK	HflC	294	281	152.59	1	3	1223.96936	3669.893528	2
		Y[M]KTPTSATR	GVIGKYT[M]DR	HflC	HflK	327	198	7.4	6	3	841.419495	2522.243931	1

[] Modified Amino Acids
* Extra miscleavage

G. Cross-linking software performance characteristics a) Total cross-link search processing time of four different software, MaxLynx (Red), MeroX (Blue), MS Annika (Green), and XlinkX (Yellow). The processing time differences for two samples' data are indicated with different tones of colors, where dark ones denote solubilized membrane samples, and light ones denote the purified protein complex. b) Number of interactions obtained from the purified protein complex and solubilized membrane shown as the unique interactions and repeating interactions. c) CPU usage of the software for the whole cross-link search process. d) RAM usage of the software for the whole cross-link search process.



H. Relative abundances of membrane and other proteins isolated from the purified protein complex (a) and solubilized membrane fraction (b).



i. Identification of the proteins in the solubilized membrane and in the purified FtsH-HflK-HflC sample.

Gene	UniProt Accession #	Gene Product	Localization	Pathway	Sample Source
Stress-induced chaperone system					
clpB	P63284	Chaperone protein ClpB	Cytoplasm	Response to heat; protein folding	Solubilized Membrane
dnaJ	P08622	Chaperone protein DnaJ	Cytoplasm	Response to heat; protein refolding	Solubilized Membrane & Purified Sample
dnaK	P0A6Y8	Chaperone protein DnaK	Cytoplasm & Cell inner membrane	Response to heat; protein refolding	Solubilized Membrane & Purified Sample
groL	P0A6F5	Chaperonin GroEL	Cytoplasm	Response to heat; protein refolding	Solubilized Membrane & Purified Sample
Metabolism and energy production					
atpA	P0ABB0	ATP synthase subunit alpha	Cell inner membrane	ATP biosynthesis	Solubilized Membrane & Purified Sample
hflX	P25519	GTPase HflX	Cytoplasm	GTP hydrolysis	Solubilized Membrane & Purified Sample
Translation					
rplD	P60723	50S ribosomal protein L4	Cytoplasm	Structural constituent of ribosome	Solubilized Membrane & Purified FtsH-HflK-HflC Complex
rplL	P0A7K2	50S ribosomal protein L7/L12	Cytoplasm	Structural constituent of ribosome	Solubilized Membrane & Purified FtsH-HflK-HflC Complex
rplU	P0AG48	50S ribosomal protein L21	Cytoplasm	Structural constituent of ribosome	Solubilized Membrane
rlmE (ftsJ, mrsF, rrmJ)	P0C0R7	Ribosomal RNA large subunit methyltransferase E	Cytoplasm	Structural constituent of ribosome	Solubilized Membrane
Degradation					
gpmA	P62707	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	Cytoplasm	Carbohydrate degradation; glycolysis	Solubilized Membrane
ATP-dependent proteolytic activity					
clpP	P0A6G7	ATP-dependent Clp protease proteolytic subunit	Cytoplasm	Proteolysis	Solubilized Membrane & Purified FtsH-HflK-HflC Complex
clpX	P0A6H1	ATP-dependent Clp protease ATP-binding subunit ClpX	Cytosol	Proteolysis	Solubilized Membrane & Purified FtsH-HflK-HflC Complex
hslU	P0A6H5	ATP-dependent protease ATPase subunit HslU	Cytoplasm	Proteolysis	Solubilized Membrane
ftsH	P0AAI3	ATP-dependent zinc metalloprotease FtsH	Cell inner membrane	Proteolysis	Solubilized Membrane & Purified FtsH-HflK-HflC Complex
Suppression					
qmcA (ybbK)	P0AA53	Protein QmcA (Identified as a multi-copy suppressor of an FtsH/HtpX protease double disruption mutant)	Cell inner membrane	Quality control-related membrane complex	Solubilized Membrane
Regulators					
hflK	P0ABC7	Modulator of FtsH protease HflK	Cell inner membrane	Regulation of proteolytic activity of FtsH	Solubilized Membrane & Purified FtsH-HflK-HflC Complex
hflC	P0ABC3	Modulator of FtsH protease HflC	Cell inner membrane	Regulation of proteolytic activity of FtsH	Solubilized Membrane & Purified FtsH-HflK-HflC Complex
Insertion					
yidC	P25714	Membrane protein insertase YidC	Cell inner membrane	Cytochrome c biogenesis (system I type)	Solubilized Membrane & Purified FtsH-HflK-HflC Complex
yajC	P0ADZ7	Sec translocon accessory complex subunit YajC	Cell inner membrane	Cytochrome c biogenesis (system I type)	Solubilized Membrane

J. The connections of FtsH, HflK and HflC with other proteins.

FtsH					
Protein 1	Gene 1	From	Protein 2	Gene 2	To
P0AAI3	ftsH	533	P21507	srmB	407
P0AAI3	ftsH	31	P21365	yciC	2
P0AAI3	ftsH	533	P10121	ftsY	2
P0AAI3	ftsH	494	P0ABB8	mgtA	350
P0AAI3	ftsH	31	P39363	sgcA	6
P0AAI3	ftsH	203	P00562	ftsY	676
P0AAI3	ftsH	332	P06960	argF	35
P0AAI3	ftsH	535	P0A7Q1	rpmI	36
P0AAI3	ftsH	332	P46837	yhgF	704
P0AAI3	ftsH	550	P23841	xapR	133
P0AAI3	ftsH	550	P76235	yeaH	222
P0AAI3	ftsH	535	P0ACV0	lpxL	191
P0AAI3	ftsH	533	P0AF20	nagC	63
P0AAI3	ftsH	550	P23367	mutL	165
P0AAI3	ftsH	265	P0A6A0	ubiB	550
P0AAI3	ftsH	332	P0AF20	nagC	63
P0AAI3	ftsH	580	P0AAB2	wzb	119
P0AAI3	ftsH	433	P0A853	tnaA	409
P0AAI3	ftsH	408	P25526	gabD	281
P0AAI3	ftsH	550	P0ACR7	yfeR	274
P0AAI3	ftsH	461	P0A7E9	pyrH	222
P0AAI3	ftsH	31	P0AG90	secD	608
P0AAI3	ftsH	332	P0AF06	motB	234
P0AAI3	ftsH	332	P0A6X1	hemA	208
P0AAI3	ftsH	76	P0AAG8	mglA	260
P0AAI3	ftsH	332	P37631	yhiN	120
P0AAI3	ftsH	332	P31548	thiQ	180
P0AAI3	ftsH	332	P18775	dmsA	134
P0AAI3	ftsH	332	P37627	yhiJ	88
P0AAI3	ftsH	332	P42632	tdcE	147
P0AAI3	ftsH	332	P0AGC3	slt	300
P0AAI3	ftsH	433	P0AA16	ompR	184
P0AAI3	ftsH	494	P0AEJ4	envZ	228
P0AAI3	ftsH	203	P37330	glcB	421
P0AAI3	ftsH	550	P0A763	ndk	1

P0AAI3	ftsH	332	P0ADR8	ppnN	337
P0AAI3	ftsH	535	P07118	valS	113
P0AAI3	ftsH	203	P51025	frmB	235
P0AAI3	ftsH	437	P31447	yidJ	408
P0AAI3	ftsH	148	P37659	bcsG	382
P0AAI3	ftsH	332	P76613	ypjC	81
P0AAI3	ftsH	332	P0A6H8	clsA	286
P0AAI3	ftsH	433	P37127	aegA	619
P0AAI3	ftsH	332	P0ABD3	bfr	53
P0AAI3	ftsH	494	P32128	yihF	207
P0AAI3	ftsH	533	P37177	ptsP	97
P0AAI3	ftsH	391	P78271	yfeS	70
P0AAI3	ftsH	433	P77561	ydeP	288
P0AAI3	ftsH	494	P37640	yhjB	160
P0AAI3	ftsH	332	Q46861	ygiQ	1
P0AAI3	ftsH	550	P0AAB4	ubiD	5
P0AAI3	ftsH	533	P28638	yhdJ	78
P0AAI3	ftsH	76	P06612	topA	3
P0AAI3	ftsH	203	P08331	cpdB	550
P0AAI3	ftsH	461	P0AFB8	glnG	445
P0AAI3	ftsH	433	P0AA99	yafK	210
P0AAI3	ftsH	332	P76573	yfgI	173
P0AAI3	ftsH	533	P15288	pepD	393
P0AAI3	ftsH	533	P52043	scpC	318
P0AAI3	ftsH	461	P37008	yagB	1
P0AAI3	ftsH	543	P60785	lepA	203
P0AAI3	ftsH	741	P18775	dmsA	580
P0AAI3	ftsH	580	P32694	yjbM	153
P0AAI3	ftsH	46	P07364	cheR	76
P0AAI3	ftsH	494	P37767	yfhH	57
P0AAI3	ftsH	494	P0ABH7	gltA	1
P0AAI3	ftsH	203	P0A9Q5	accD	84
P0AAI3	ftsH	203	P69931	hda	232
P0AAI3	ftsH	535	P13009	metH	446
P0AAI3	ftsH	84	P37659	bcsG	382
P0AAI3	ftsH	136	P0A9Q5	accD	84
P0AAI3	ftsH	76	P21177	fadB	302
P0AAI3	ftsH	203	P0A9Q5	accD	84

P0AAI3	ftsH	203	Q47152	rayT	41
P0AAI3	ftsH	203	P37671	yiaJ	1
P0AAI3	ftsH	433	Q47688	ykfC	0
P0AAI3	ftsH	433	P0ABK9	nrfA	90
P0AAI3	ftsH	408	P24175	manB	0
P0AAI3	ftsH	408	P76542	intZ	106
P0AAI3	ftsH	433	P0AA10	rplM	111
P0AAI3	ftsH	203	P37645	yhjG	380
P0AAI3	ftsH	408	P37009	fbpC	0
P0AAI3	ftsH	533	P02929	tonB	197
P0AAI3	ftsH	408	Q47688	ykfC	0
P0AAI3	ftsH	494	P0AAJ8	hybA	215
P0AAI3	ftsH	332	P0ABN5	dcuA	220
P0AAI3	ftsH	332	P0AAJ8	hybA	215
P0AAI3	ftsH	550	Q46890	otnC	0
P0AAI3	ftsH	433	P52696	ybhD	61
P0AAI3	ftsH	408	P76205	arpB	1
P0AAI3	ftsH	332	P42592	ygjK	546
P0AAI3	ftsH	199	P0A993	fbp	433
P0AAI3	ftsH	408	P39285	mscM	67
P0AAI3	ftsH	332	P0ADE6	kbp	13
P0AAI3	ftsH	533	P76041	ycjM	1
P0AAI3	ftsH	408	P26266	fepE	228
P0AAI3	ftsH	433	P0A7J7	rplK	97

HflK					
Protein 1	Gene 1	From	Protein 2	Gene 2	To
P0ABC7	hflK	294	P37650	bcsC	702
P0ABC7	hflK	172	P77694	ecpD	27
P0ABC7	hflK	198	Q47538	tauB	123
P0ABC7	hflK	198	Q46868	ubiK	6
P0ABC7	hflK	198	P37652	bcsB	424
P0ABC7	hflK	294	P0A8P8	xerD	136
P0ABC7	hflK	294	P33997	alpA	2
P0ABC7	hflK	294	P43329	hrpA	1262
P0ABC7	hflK	294	Q46814	xdhD	188
P0ABC7	hflK	333	P0DMC5	rcsC	502
P0ABC7	hflK	198	P0AB65	yccX	63
P0ABC7	hflK	294	P76641	guaD	9
P0ABC7	hflK	198	P0AAG3	gltL	228
P0ABC7	hflK	198	A0A7H2C793	yqiM	1
P0ABC7	hflK	31	P37024	hrpB	784
P0ABC7	hflK	198	P24183	fdnG	1
P0ABC7	hflK	198	P60757	hisG	2
P0ABC7	hflK	310	P63224	gmhA	147
P0ABC7	hflK	198	P0C093	slmA	0
P0ABC7	hflK	198	P0ADP9	yihD	0

HflC					
Protein 1	Gene 1	From	Protein 2	Gene 2	To
P0ABC3	hflC	137	P76594	patZ	526
P0ABC3	hflC	137	P0AG07	rpe	9/12
P0ABC3	hflC	137	P0AFX7	rseA	17
P0ABC3	hflC	333	P68767	pepA	156
P0ABC3	hflC	137	P03018	uvrD	621
P0ABC3	hflC	85	P75785	opgE	8
P0ABC3	hflC	44	P23367	mutL	159
P0ABC3	hflC	137	P09126	hemD	2
P0ABC3	hflC	327	P68767	pepA	156
P0ABC3	hflC	137	P05791	ilvD	3
P0ABC3	hflC	137	P00363	frdA	324
P0ABC3	hflC	137	P77180	ykgH	109
P0ABC3	hflC	327	P42592	ygjK	189
P0ABC3	hflC	36	P0A7A7	plsB	446
P0ABC3	hflC	327	P15038	helD	4
P0ABC3	hflC	137	P17445	betB	477
P0ABC3	hflC	137	P08956	hsdR	1124
P0ABC3	hflC	327	P76093	ynbD	301
P0ABC3	hflC	36	P22939	ispA	262
P0ABC3	hflC	137	P45537	yhfK	300
P0ABC3	hflC	137	P0A6F5	groEL	15
P0ABC3	hflC	137	P06710	dnaX	571
P0ABC3	hflC	137	P77611	rsxC	258
P0ABC3	hflC	137	P07017	tar	413
P0ABC3	hflC	137	A0A385XJN2	ygiA	64
P0ABC3	hflC	327	P00452-2;P00452	nrdA	359;384
P0ABC3	hflC	137	P76235	yeaH	14
P0ABC3	hflC	36	Q46908	ygcR	204
P0ABC3	hflC	137	P45800	yrfF	446
P0ABC3	hflC	137	P06136	ftsQ	120
P0ABC3	hflC	137	P23842	pdeA	690
P0ABC3	hflC	36	P0AF36	zapB	61
P0ABC3	hflC	44	P0A8U2	yafD	3
P0ABC3	hflC	327	P31064	yedE	189
P0ABC3	hflC	137	P18776;P0AAJ1	dmsB;ynfG	2;2
P0ABC3	hflC	137	P0AEZ7	mltD	233
P0ABC3	hflC	36	P0ABG4	ftsW	167
P0ABC3	hflC	137	P77551;P75719	rzpR;rzpD	21;76
P0ABC3	hflC	137	P39375	iraD	17
P0ABC3	hflC	122	P0AEW6	gsk	10

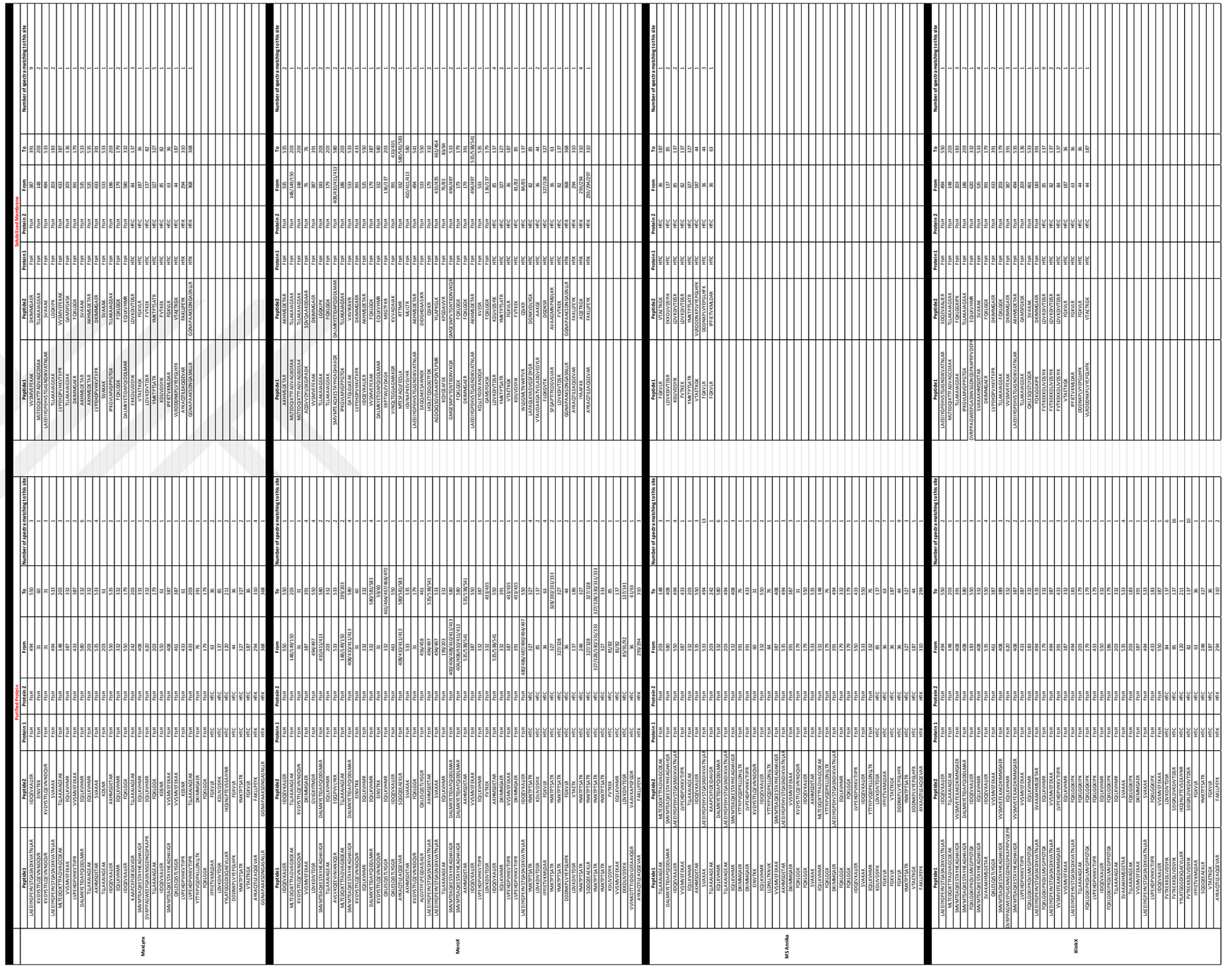
P0ABC3	hflC	327	P00350	gnd	314
P0ABC3	hflC	327	P15043	recQ	593
P0ABC3	hflC	137	P30128	greB	15
P0ABC3	hflC	327	P45463	ttdR	91
P0ABC3	hflC	327	P15082	srlR	2
P0ABC3	hflC	122	P64554	queE	46
P0ABC3	hflC	137	P39452	nrdE	524
P0ABC3	hflC	44	P21888	cysS	370
P0ABC3	hflC	137	P37682	yiaU	1
P0ABC3	hflC	137	P75972	ymfI	1
P0ABC3	hflC	36	P0ADY3	rplN	1
P0ABC3	hflC	36	P0CK95	yghJ	167
P0ABC3	hflC	122	P0A8K1	psd	6
P0ABC3	hflC	137	P0A8M0	asnS	1
P0ABC3	hflC	36	A0A7H2C767;P63284	clpB	303;451
P0ABC3	hflC	248	P32128	yihF	350
P0ABC3	hflC	137	P63389	yheS	272
P0ABC3	hflC	137	P04036	dapB	187
P0ABC3	hflC	36	P12282	moeB	1
P0ABC3	hflC	137	P77615	ycjW	317
P0ABC3	hflC	36	P16703	cysM	88
P0ABC3	hflC	137	P37766	ydiF	455
P0ABC3	hflC	36	P25519	hflX	391
P0ABC3	hflC	36	P77610	ansP	15
P0ABC3	hflC	137	P0AAV0	ybgE	14
P0ABC3	hflC	137	P23890	cadC	463
P0ABC3	hflC	44	P0A6F1	carA	2
P0ABC3	hflC	44	P0AC75	waaA	168
P0ABC3	hflC	137	P0AF28	narL	188
P0ABC3	hflC	36	P33596	recX	1
P0ABC3	hflC	137	P64448	ynbE	37
P0ABC3	hflC	137	P0AGL5	ratA	2
P0ABC3	hflC	36	P27829	wecC	172
P0ABC3	hflC	36	P0AAF3	araG	324
P0ABC3	hflC	36	P37773	mpl	198
P0ABC3	hflC	327	Q46927	tcdA	259
P0ABC3	hflC	36	P24188	trhO	183
P0ABC3	hflC	36	P21170	speA	1
P0ABC3	hflC	137	P19323	fhlA	526
P0ABC3	hflC	137	P0A6N4	efp	54
P0ABC3	hflC	137	P0ADK0	yiaF	0
P0ABC3	hflC	44	P30750	metN	0

P0ABC3	hflC	137	P0DSF4	ynfU	8
P0ABC3	hflC	44	P0AGM0	yhhT	8
P0ABC3	hflC	44	P77306	yqiK	378
P0ABC3	hflC	82	P0AF78	yjfJ	44
P0ABC3	hflC	137	P77488	dxs	284
P0ABC3	hflC	327	P0AEF0	dnaC	1
P0ABC3	hflC	44	P0A9Q7	adhE	876
P0ABC3	hflC	137	P0A749	murA	152
P0ABC3	hflC	137	P32721	alsA	338
P0ABC3	hflC	122	P0ADP9	yihD	0
P0ABC3	hflC	137	P29745	pepT	177
P0ABC3	hflC	137	P71311	yaiS	3
P0ABC3	hflC	248	P09184	vsr	92
P0ABC3	hflC	85	P0AFG8	aceE	881
P0ABC3	hflC	327	P21365	yciC	0
P0ABC3	hflC	84	P0AES4	gyrA	1

K. Complex inter-protein interactions.

	Purified Protein							Solubilized Membrane						
	Peptide1	Peptide2	Protein 1	Protein 2	From	To	Number of spectra matching to this site	Peptide1	Peptide2	Protein 1	Protein 2	From	To	Number of spectra matching to this site
MaxLynx	VTNPEKYLYSVTSPDDSLR	FGKVLR	HflK	HflC	172	36	1	YYLATGGGDISQAEVLLKR	KDSNR	HflC	FtsH	120	61	1
	VLVNDKGGNLMVLPLDQMLK	YMKTPTSATR	HflK	HflC	346	327	6	AYKAQTILEAQGEVAR	SQGQEEAEKLR	HflK	HflC	294	248	3
	VTNPEKYLYSVTSPDDSLR	KFSDR	HflK	HflC	172	122	1	VLVNDKGGNLMVLPLDQMLK	YMKTPTSATR	HflK	HflC	346	327	1
	AYKAQTILEAQGEVAR	YMKTPTSATR	HflK	HflC	294	327	2	LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	137	198	4
	KVDYSTFLQEVNNDQVR	PTFIDEVKPVNVEAVR	FtsH	HflK	31	133	2							
	LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	137	198	3							
	GEGDAEAAKLFADAFSK	AYKAQTILEAQGEVAR	HflC	HflK	281	294	1							
	YYLATGGGDISQAEVLLKR	LLDNLLTKNVK	HflC	FtsH	120	84	1							
	IKQINLPTEVSEAIYNR	KDSNR	HflC	FtsH	211	61	1							
	IKQINLPTEVSEAIYNR	EINVTKK	HflC	FtsH	211	60	1							
	KVDYSTFLQEVNNDQVR	IPFIETVKMLDAR	FtsH	HflC	31	63	6							
	Peptide1	Peptide2	Protein 1	Protein 2	From	To	Number of spectra matching to this site	Peptide1	Peptide2	Protein 1	Protein 2	From	To	Number of spectra matching to this site
MeroX	KVDYSTFLQEVNNDQVR	IPFIETVKMLDAR	FtsH	HflC	31	63	1	GEGDAEAAKLFADAFSK	FAKLLPEYK	HflC	HflK	281	310	1
	QYLIAPSILSADFAR	LDVKDIVTDSR	FtsH	HflC	31	61/63	2	AYKAQTILEAQGEVAR	SQGQEEAEKLR	HflC	HflK	248	293/294	1
	SEIGRLDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	137	198/199/200	10	DATNKAADIVLQAAIAAGAPK	IPLAKMAVAESGMGIVEDK	HflC	HflK	137	198	2
	LAEEIIYGPEHVSTGASNDIKVATNLAR	IIDQEVKALIER	HflC	HflK	137	198	1	VLVNDKGGNLMVLPLDQMLK	YMKTPTSATR	HflC	HflK	327	346	2
	LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	141/143	198	4	LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	141	198	1
	SMVMTEAQKESTAYHEAGHAIIGR	DALMKYETIDAPQIDDLMAR	HflC	HflK	248	293/294	1	WLGGMLTNWKTVR	QSIKR	HflC	HflK	248	294	1
	LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	137/141/143	198/199/200	1							
	KNGKEVQKLTGK	EQILKVHMR	HflK	HflC	294	328/330/331/333	1							
	Peptide1	Peptide2	Protein 1	Protein 2	From	To	Number of spectra matching to this site	Peptide1	Peptide2	Protein 1	Protein 2	From	To	Number of spectra matching to this site
MS Annika	IPFIETVKMLDAR	KVDYSTFLQEVNNDQVR	HflC	FtsH	63	31	3	GVIGKYTMDR	LDVKDIVTDSR	HflK	HflC	198	137	3
	FGKVLR	KVDYSTFLQEVNNDQVR	HflC	FtsH	36	31	1	FAKLLPEYK	GEGDAEAAKLFADAFSK	HflK	HflC	310	281	3
	EINVTKK	YYLATGGGDISQAEVLLKR	FtsH	HflC	60	120	1							
	LLDNLLTKNVK	YYLATGGGDISQAEVLLKR	FtsH	HflC	84	120	1							
	EINVTKK	IKQINLPTEVSEAIYNR	FtsH	HflC	60	211	2							
	GVIGKYTMDR	LDVKDIVTDSR	HflK	HflC	198	137	15							
	YMKTPTSATR	VLVNDKGGNLMVLPLDQMLK	HflC	HflK	327	346	6							
	SQGQEEAEKLR	AYKAQTILEAQGEVAR	HflC	HflK	248	294	1							
	KFSDR	VTNPEKYLYSVTSPDDSLR	HflC	HflK	122	172	1							
	FGKVLR	VTNPEKYLYSVTSPDDSLR	HflC	HflK	36	172	2							
	Peptide1	Peptide2	Protein 1	Protein 2	From	To	Number of spectra matching to this site	Peptide1	Peptide2	Protein 1	Protein 2	From	To	Number of spectra matching to this site
XlinkX	AYKAQTILEAQGEVAR	SQGQEEAEKLR	HflK	HflC	294	248	2	AYKAQTILEAQGEVAR	GEGDAEAAKLFADAFSK	HflK	HflC	294	281	2
	AYKAQTILEAQGEVAR	GEGDAEAAKLFADAFSK	HflK	HflC	294	281	1	AYKAQTILEAQGEVAR	SQGQEEAEKLR	HflK	HflC	294	248	1
	VLVNDKGGNLMVLPLDQMLK	YMKTPTSATR	HflK	HflC	346	327	2	LDVKDIVTDSR	GVIGKYTMDR	HflC	HflC	137	198	4
	LDVKDIVTDSR	GVIGKYTMDR	HflC	HflK	137	198	19	GEGDAEAAKLFADAFSK	FAKLLPEYK	HflC	HflK	281	310	1
	FVTKEKKDLIVDSYIK	GVIGKYTMDR	HflC	HflK	82	198	2							
	FVTKEKKDLIVDSYIK	GVIGKYTMDR	HflC	HflK	84	198	1							
	YYLATGGGDISQAEVLLKR	LLDNLLTKNVK	HflC	FtsH	120	84	1							
	KVDYSTFLQEVNNDQVR	IPFIETVKMLDAR	FtsH	HflC	31	63	1							

L. Complex intra-protein interactions.



M. Interactions of complex proteins with other proteins.

	Purified Protein							Solubilized Membrane						
	Peptide 1	**Peptide 2**	**Protein 1**	**Protein 2**	**From**	**To**	**Score**	**Peptide 1**	**Peptide 2**	**Protein 1**	**Protein 2**	**From**	**To**	**Score**
	SVAKAK	KAKEK	P0AAI3	P21507	533	407	104.42	TLLAKAIAGEAK	DVMRKLVILAR	P0AAI3	P00562	203	676	62.27
	KVDYSTFLQEVNNDQVR	SITAQSVYR	P0AAI3	P21365	31	2	90.123							
	SVAKAK	AKEKK	P0AAI3	P10121	533	2	86.18							
MaxLynx	SILVTRPSPAGEELVSRLR	LDVKDIVTDSR	P09126	P0ABC3	2	137	84.076							
	LAEEIIYGPEHVSTGASNDIKVATNLAR	LSKQK	P0AAI3	P0ABB8	494	350	65.712							
	LIPQAKSQPPSMESAMVLR	AYKAQTILEAQGEVAR	P37650	P0ABC7	702	294	64.862							
	KVDYSTFLQEVNNDQVR	MINDIKWVQAQR	P0AAI3	P39363	31	6	44.706							
	YMKTPTSATR	KMVFNVPTR	P0ABC3	P68767	327	156	42.806							
	Peptide 1	**Peptide 2**	**Protein 1**	**Protein 2**	**From**	**To**	**Score**	**Peptide 1**	**Peptide 2**	**Protein 1**	**Protein 2**	**From**	**To**	**Score**
	LRSPDIPHKSEVQGVMLYLR	LDVKDIVTDSR	P0ABC3	P76594	137	526	113	ITDKQER	MLNPIVR	P0ABC3	P03018	137	621	111
	KVDYSTFLQEVNNDQVR	IPFIETVKMLDAR	P0AG07	P0ABC3	9/12	137	88							
	AYKAQTILEAQGEVAR	YMKTPTSATR	P06960	P0AAI3	35	332	87							
	LDVKDIVTDSR	RLAQHPYDR	P0AFX7	P0ABC3	17	137	86							
MeroX	YMKTPTSATR	KMVFNVPTR	P68767	P0ABC3	156	333	77							
	AKHMSDETAR	KATKR	P0A7Q1	P0AAI3	36	535	72							
	HLNNRGAQQKLAEALSMLK	LDVKDIVTDSR	P0ABC3	P06710	137	571	67							
	LDVKDIVTDSR	RLYGK	P0ABC3	P03018	137	621	64							
	KDLVDSYIK	ESLVTRSR	P75785	P0ABC3	8	85	57							
	DDDNKPLVYEPGLHFK	RKFLR	P0ABC3	P23367	44	159	49							
MS Annika	**Peptide 1**	**Peptide 2**	**Protein 1**	**Protein 2**	**From**	**To**	**Score**	**Peptide 1**	**Peptide 2**	**Protein 1**	**Protein 2**	**From**	**To**	**Score**
	SITAQSVYR	KVDYSTFLQEVNNDQVR	P21365	P0AAI3	2	31	223.9	YEKDDSIRK	YMKTPTSATR	P00452-2;P00452	P0ABC3	359;384	327	88.69
	AAVSKTTWADAPAR	VTNPEKYLYSVTSPDDSLR	P77694	P0ABC7	27	172	168.11	MPKYR	LDVKDIVTDSR	P05791	P0ABC3	3	137	41.93
	EQILKVHMR	VKVLEVDLQRK	P0AAI3	P46837	332	704	141.01	KLHER	LDVKDIVTDSR	P00363	P0ABC3	324	137	26.42
	LDVKDIVTDSR	LEWSGDKRALPTAEEEISR	P0ABC3	P77180	137	109	135.38							
	YMKTPTSATR	TIAGEYPDYQRKISATR	P0ABC3	P42592	327	189	133.93							
	DLKKMVADR	LDVKDIVTDSR	P19323	P0ABC3	526	137	130.61							
	FGKVLR	GATKEKESLPQMLR	P0ABC3	P0A7A7	36	446	127.42							
	IIDQEVKALIER	FLRENPNVDVLFREKMPAMQMALLER	P0AAI3	P23841	550	133	126.06							
	YMKTPTSATR	MELKATTLGKRLAQHPYDR	P0ABC3	P15038	327	4	126.04							
	LDVKDIVTDSR	ENGVMTLQSYTQVKSIQVEMAK	P0ABC3	P17445	137	477	124.86							
	VGLEGAEKR	QATDSALRGVIGKYTMDR	Q47538	P0ABC7	123	198	123.05							
	LDVKDIVTDSR	EKVVLDDDVFKTGNFHRR	P0ABC3	P08956	137	1124	120.75							
	YMKTPTSATR	LEPVSKMTAGVYLGAFPR	P0ABC3	P76093	327	301	111.76							
	FGKVLR	KARDIDDAR	P0ABC3	P22939	36	262	106.53							
	EIAELRAKIER	IIDQEVKALIER	P76235	P0AAI3	222	550	103.79							
	AKHMSDETAR	KGEVVWYAPDHDYGPR	P0AAI3	P0ACV0	535	191	103.56							
	SVAKAK	GLIKEVDQQASTGGR	P0AAI3	P0AF20	533	63	102.56							
	LDVKDIVTDSR	LPTRFTMEKQIGALEKIAR	P0ABC3	P45537	137	300	98.23							
	LDVKDIVTDSR	DVKFGNDARVKMLR	P0ABC3	P0A6F5	137	15	95.15							
	LDVKDIVTDSR	HLNNRGAQQKLAEALSMLK	P0ABC3	P06710	137	571	91.42							
	LDVKDIVTDSR	QLTYILTGKQVPHGGR	P0ABC3	P77611	137	258	90.82							
	LDVKDIVTDSR	NLASRSAQAAKEIKALIEDSVSR	P0ABC3	P07017	137	413	89.52							
	IIDQEVKALIER	TEKTEFNHIDEIIR	P0AAI3	P23367	550	165	87.92							
	GVIGKYTMDR	KIEQIARQVHESMPKGIR	P0ABC7	Q46868	198	6	87.87							
	GVIGKYTMDR	YTMPPVKDSSR	P0ABC7	P37652	198	424	87.66							
	LDVKDIVTDSR	IHTPRIVPQKLERTPSDTSR	P0ABC3	A0A385XJN2	137	64	85.39							
	NGTNMKLLAER	IIDQEVKALIER	P0A6A0	P0AAI3	265	550	83.76							
	EQILKVHMR	GLIKEVDQQASTGGR	P0AAI3	P0AF20	332	63	82.98							
	YEKDDSIRK	YMKTPTSATR	P00452-2;P00452	P0ABC3	359;384	327	82.43							
	NKSMVNR	LDVKDIVTDSR	P76235	P0ABC3	14	137	80.95							
	AYKAQTILEAQGEVAR	DKAMLEVLYATGLRVSELVGLTMSDISLR	P0ABC7	P0A8P8	294	136	80.53							
	DALMKYETIDAPQIDDLMAR	VMLFGHWDNECEIPDPYRKSR	P0AAI3	P0AAB2	580	119	80.33							
	FGKVLR	MAAGKAEIIR	P0ABC3	Q46908	36	204	78.62							
	LVPEHDPVHKVTIIPR	DPKTGKQLPCPAELLRLTIPR	P0AAI3	P0A853	433	409	77.67							
	LDVKDIVTDSR	SLPLPESELVNKATALTEAVNR	P0ABC3	P45800	137	446	77.37							
	NNRSAVR	AYKAQTILEAQGEVAR	P33997	P0ABC7	2	294	76.04							
	AVEGALASKFRNAGQTCVCANR	SMVMTEAQKESTAYHEAGHAIIGR	P25526	P0AAI3	281	408	75.92							
	LDVKDIVTDSR	QWPDELKIHLVEYVPIAR	P0ABC3	P06136	137	120	75.73							
	LDVKDIVTDSR	AKSLSVVAEFVETQQQQALLHK	P0ABC3	P23842	137	690	75							
	REDEDVKEIR	AYKAQTILEAQGEVAR	P43329	P0ABC7	1262	294	74.98							
	MVQAKPCYVEDR	AYKAQTILEAQGEVAR	Q46814	P0ABC7	188	294	74.93							
	QLMLVRRKNR	IIDQEVKALIER	P0ACR7	P0AAI3	274	550	74.73							
	FGKVLR	ENNHLKEQQNGWQER	P0ABC3	P0AF36	36	61	73.61							
	DDDNKPLVYEPGLHFK	RKNTYAMRYVAGQPAER	P0ABC3	P0A8U2	44	3	72.63							
	ELPKGVDR	LYIETMEKVLGNTR	P0DMC5	P0ABC7	502	333	72.61							
	YMKTPTSATR	MQKVSAASPLTQKPDQAR	P0ABC3	P31064	327	189	72.57							
	LDVKDIVTDSR	TTQYGFFIDSSR	P0ABC3	P18776;P0AAJ1	137	2;2	72.28							
	VFNMNKPGALR	QKLESQISTLYGGR	P0A7E9	P0AAI3	222	461	72.16							
	AIVNLLYGGKR	KVDYSTFLQEVNNDQVR	P0AG90	P0AAI3	608	31	72.13							
	EQILKVHMR	RELMVGGLDSGKVLRVVGMAATMR	P0AAI3	P0AF06	332	234	70							
	LDVKDIVTDSR	QYVPKMLALSDILKNSKR	P0ABC3	P0AEZ7	137	233	68.38							
	KGDEVR	FGKVLR	P0ABG4	P0ABC3	167	36	65.62							
	EQILKVHMR	VQKMIIANRTR	P0AAI3	P0A6X1	332	208	65.23							
	LDVKDIVTDSR	DVAALDAKYTKELADAKAENDALR	P0ABC3	P77551;P75719	137	21;76	64.49							
	ENKPGEVILEVR	YTTYIPVQDPKLLDNLLTK	P0AAG8	P0AAI3	260	76	64							
	LDVKDIVTDSR	MRQSLQAVLPEISGNKTSSLR	P0ABC3	P39375	137	17	63.18							
	EQILKVHMR	TLGQLFCDDSAQQIVDMLVDECEKGNVTFR	P0AAI3	P37631	332	120	62.43							
	EQILKVHMR	QEMLTLVSTSCQQQKMTLLMVSHSVEDAAR	P0AAI3	P31548	332	180	61.3							
	KFSDR	KSKHYFPVNAR	P0ABC3	P0AEW6	122	10	60.29							
	EQILKVHMR	GEGKFERISWEEAYDIIATNMQR	P0AAI3	P18775	332	134	59.92							
	YMKTPTSATR	VLSGPQAQPAGDKAEFIEK	P0ABC3	P00350	327	314	57.56							
	YMKTPTSATR	LERFGKPFMALIR	P0ABC3	P15043	327	593	57.08							
	LDVKDIVTDSR	LKQELNYLWREEP0AG07VTKK	P0ABC3	P30128	137	15	56.31							
	EQILKVHMR	FQQSIQDDKISFTIQTDHSEKYSMLSGMR	P0AAI3	P37627	332	88	55.84							
	EQILKVHMR	KTHNQGVFDVYSPDMLRCR	P0AAI3	P42632	332	147	55.72							
	EQILKVHMR	LMGNDVTDEQAKWRDDAIMR	P0AAI3	P0AGC3	332	300	55.16							
	YMKTPTSATR	LVDDVTQIKTP0AG07GMIR	P0ABC3	P45463	327	91	55.06							
	KPRQR	YMKTPTSATR	P15082	P0ABC3	2	327	54.14							
	KFSDR	LQGCPVGCAWCDTKHTWEKLEDR	P0ABC3	P64554	122	46	53.61							
	EPLSRDKLMNLAR	LVPEHDPVHKVTIIPR	P0AA16	P0AAI3	184	433	53.61							
	KMVFNVPTR	YMKTPTSATR	P68767	P0ABC3	156	327	51.99							
	AFNHMAAGVKQLADDR	LAEEIIYGPEHVSTGASNDIKVATNLAR	P0AEJ4	P0AAI3	228	494	51.44							
	TLLAKAIAGEAK	IETMLGMAPNTLKMGIMDEER	P0AAI3	P37330	203	421	51.36							
	GETFAGFKQSR	LDVKDIVTDSR	P39452	P0ABC3	524	137	50.88							
	LKAEDMAAANAMASHLR	DDDNKPLVYEPGLHFK	P21888	P0ABC3	370	44	50.64							
	KGMAYITESR	LDVKDIVTDSR	P32721	P0ABC3	338	137	50.1							
	MTKLQLKYR	LDVKDIVTDSR	P37682	P0ABC3	1	137	50.04							
	MAIER	IIDQEVKALIER	P0A763	P0AAI3	1	550	49.91							
	MNQFYVHVR	LDVKDIVTDSR	P75972	P0ABC3	1	137	49.8							
	FGKVLR	MIQEQTMLNVADNSGAR	P0ABC3	P0ADY3	36	1	49.44							
	FGKVLR	VSFSLEDAQELAGSDDKK	P0ABC3	P0CK95	36	167	49.17							
	EQILKVHMR	SMPLVKENRRDTGDAYSFNWSMR	P0AAI3	P0ADR8	332	337	48.81							
	AKHMSDETAR	HDYGREAFIDKIWEWKAESGGTITR	P0AAI3	P07118	535	113	48.8							

MS Annika	KFSDR	MLNSFKLSLQYILPKLWLTR	P0ABC3	P0A8K1	122	6	48.7									
	GVIGKYTMDR	LMQWLKSGGPR	P0ABC7	P0AB65	198	63	48.54									
	TLLAKAIAGEAK	TPNLEKICQEMNIK	P0AAI3	P51025	203	235	48.4									
	SKMHDALLDYMDKIRDPFR	SMVMTEAQKESTAYHEAGHAIIGR	P31447	P0AAI3	437	408	48.15									
	LDVKDIVTDSR	MSVVPVADVLQGRVAVDSEVTVR	P0ABC3	P0A8M0	137	1	47.53									
	DKNSR	MLTEDQIKTTFADVAGCDEAK	P37659	P0AAI3	382	148	47.52									
	EQILKVHMR	MMSECIYQEGNAFVIMGAGEQLKR	P0AAI3	P76613	332	81	47.43									
	FGKVLR	LDMLNEELSDKER	P0ABC3	A0A7H2C767;P63284	36	303;451	47.29									
	SEPTIKQPVR	SQGQEEAEKLR	P32128	P0ABC3	350	248	47.08									
	ATKAKQAQSR	LDVKDIVTDSR	P63389	P0ABC3	272	137	47.03									
	EQILKVHMR	MEGPIATAMGIIYSCDWEIETGKR	P0AAI3	P0A6H8	332	286	46.75									
	DLKDCAVYSR	LDVKDIVTDSR	P04036	P0ABC3	187	137	45.98									
	FGKVLR	MAELSDQEMLR	P0ABC3	P12282	36	1	45.65									
	MMSGEHTLKAVR	AYKAQTILEAQGEVAR	P76641	P0ABC7	9	294	44.6									
	YQTTNPKIFAGGDAVR	LVPEHDPVHKVTIIPR	P37127	P0AAI3	619	433	44.22									
	EQILKVHMR	LNDVEYHESIDEMKHADRYIER	P0AAI3	P0ABD3	332	53	44.13									
	VTAKSFTIDSLAR	LAEEIIYGPEHVSTGASNDIKVATNLAR	P32128	P0AAI3	207	494	43.91									
	SVAKAK	LAEPINLADAQKHPSFKYIPSVKEER	P0AAI3	P37177	533	97	43.76									
	DKIMMGAER	GYQEDPKFNFMDR	P0AAI3	P78271	391	70	43.66									
	LDVKDIVTDSR	LISGKPSPQNITVATHMTLK	P0ABC3	P77615	137	317	43.21									
	GVIGKYTMDR	DAFFDDPKSDR	P0ABC7	P0AAG3	198	228	42.99									
	FGKVLR	MKLLMPDNMSQER	P0ABC3	P16703	36	88	42.85									
	LDVKDIVTDSR	FIRELPEITFSGKIALER	P0ABC3	P37766	137	455	42.82									
	FGKVLR	FYQLQAIEKEWMEEDGSVSLQVRMPIVDWR	P0ABC3	P25519	36	391	42.38									
	IGGDMALLKGMMR	LVPEHDPVHKVTIIPR	P77561	P0AAI3	288	433	41.16									
	FGKVLR	HDTDTSDQHAAKR	P0ABC3	P77610	36	15	40.69									
	EILTMLAAGESNKEIGR	LAEEIIYGPEHVSTGASNDIKVATNLAR	P37640	P0AAI3	160	494	39.29									
	EQILKVHMR	MSSISLIQPDR	P0AAI3	Q46861	332	1	37.22									
	LDVKDIVTDSR	MSKIIATLYAVMDKRPLR	P0ABC3	P0AAV0	137	14	35.64									
	MDAMKYNDLR	IIDQEVKALIER	P0AAB4	P0AAI3	5	550	35.09									
	MLMYQTRR	GVIGKYTMDR	A0A7H2C793	P0ABC7	1	198	34.57									
	VYEMKGMNR	LDVKDIVTDSR	P23890	P0ABC3	463	137	33.99									
	IKSALLVLEDGTQFHGR	DDDNKPLVYEPGLHFK	P0A6F1	P0ABC3	2	44	33.36									
	SVAKAK	VLKKQGSMYIMNSTENMPFIDLQCR	P0AAI3	P28638	533	78	32.75									
	IIDQEVKALIER	LIGVLSVGKPNAAMAPVIKRSER	P0AAI3	P08401	550	168	31.78									
	GKALVIVESPAK	YTTYIPVQDPKLLDNLLTK	P06612	P0AAI3	3	76	31.65									
	LGKFVR	DDDNKPLVYEPGLHFK	P0AC75	P0ABC3	168	44	30.42									
	LDVKDIVTDSR	LDITESTVKVHVKHMLKK	P0ABC3	P0AF28	137	188	30.38									
	FGKVLR	MTESTSR	P0ABC3	P33596	36	1	28.13									
	EMKGR	DPWGSSKPGGNSEGNGNKGGR	P37024	P0ABC7	784	31	27.64									
	LDVKDIVTDSR	EPITINMNVKIEHEIIIK	P0ABC3	P64448	137	37	27.49									
	MDVSR	GVIGKYTMDR	P24183	P0ABC7	1	198	27.27									
	TLLAKAIAGEAK	AYGGKFAGTGDSHIAFASPDENR	P0AAI3	P08331	203	550	26.61									
	LDVKDIVTDSR	ILFVGFLLMEIVMPQISR	P0ABC3	P0AGL5	137	2	25.22									
	HTQGHKQEAAR	QKLESQISTLYGGR	P0AFB8	P0AAI3	445	461	24.38									
	KPPTVSVVNGR	LVPEHDPVHKVTIIPR	P0AA99	P0AAI3	210	433	24.33									
	EQILKVHMR	AEENKSDSWFN	P0AAI3	P76573	332	173	21.67									
	FGKVLR	VLPGQVMVELIKNDR	P0ABC3	P27829	36	172	21.16									
	SVAKAK	LAGAKTEAKGAYPGWQPDANSPVMHLVR	P0AAI3	P15288	533	393	20.25									
	SVAKAK	KIYDNMDYFASR	P0AAI3	P52043	533	318	20.2									
	MNKDSTQTWGLK	QKLESQISTLYGGR	P37008	P0AAI3	1	461	19.76									
	FGKVLR	KPSHAIAAGMMLCPEDR	P0ABC3	P0AAF3	36	324	19.23									
	FGKVLR	FVHYCPRTULNNLEFDHADIFDDLKAIQK	P0ABC3	P37773	36	198	18.2									
	STVKQLR	TLLAKAIAGEAK	P60785	P0AAI3	543	203	17									
	KMMAKAARQG	YMKTPTSATR	Q46927	P0ABC3	259	327	16.87									
	FGKVLR	EQLPKAVEMMQAHKDK	P0ABC3	P24188	36	183	16.48									
	TDNTR	GVIGKYTMDR	P60757	P0ABC7	2	198	15.85									
	RGIHNGDKVR	DALMKYETIDAPQIDDLMAR	P18775	P0AAI3	741	580	15.08									
	DALMKYETIDAPQIDDLMAR	KYLGEMKRLADGGEMNFDGYLDK	P0AAI3	P32694	580	153	14.36									
	AGIVLADHKR	YTTYIPVQDPKLLDNLLTK	P07364	P0AAI3	46	76	14.3									
	FAQKLGYK	LAEEIIYGPEHVSTGASNDIKVATNLAR	P37767	P0AAI3	57	494	14.06									
	MADTKAKLTLNGDTAVELDVLK	LAEEIIYGPEHVSTGASNDIKVATNLAR	P0ABH7	P0AAI3	1	494	10.88									
	FGKVLR	MSDDMSMGLPSSAGEHGVLR	P0ABC3	P21170	36	1	6.44									
	Peptide 1	**Peptide 2**	**Protein 1**	**Protein 2**	**From**	**To**	**Score**	**Peptide 1**	**Peptide 2**	**Protein 1**	**Protein 2**	**From**	**To**	**Score**		
	TLLAKAIAGEAK	DVLKFR	P0AAI3	P0A9Q5	203	84	56.47	LTIPFVKEILKL	TLLAKAIAGEAK	P69931	P0AAI3	232	203	69.38		
	LDVKDIVTDSR	DLKKMVADR	P0ABC3	P19323	137	526	48.17	VPIMIDSSKWDVIEKGLKCIQGK	AKHMSDETAR	P13009	P0AAI3	446	535	51.77		
	LVPEHDPVHKVTIIPR	MIISEMQRK	P0AAI3	Q47688	433	0	45.63	LDVKDIVTDSR	VEKTFK	P0ABC3	P0A6N4	137	54	45.03		
	LDVKDIVTDSR	MATGKSCSR	P0ABC3	P0ADK0	137	0	45.63	EKGMKVITLTGKDGGK	FAKLLPEYK	P63224	P0ABC7	147	310	36.42		
	DDDNKPLVYEPGLHFK	MIKLSNITK	P0ABC3	P30750	44	0	45.63	LLDNLLTKNVK	DKNSR	P0AAI3	P37659	84	382	36.01		
	LVILWAGYPFSRDYNKPR	LVPEHDPVHKVTIIPR	P0ABK9	P0AAI3	90	433	41.28	GAMSFGKSK	DVLKFR	P0AAI3	P0A9Q5	136	84	34.81		
	SMVMTEAQKESTAYHEAGHAIIGR	MKKLTCFKAYDIR	P0AAI3	P24175	408	0	39.02	YTTYIPVQDPKLLDNLLTK	GKAKKLTK	P0AAI3	P21177	76	302	30.67		
	LDVKDIVTDSR	SERKNSKSR	P0ABC3	P0DSF4	137	8	38.82	TLLAKAIAGEAK	DVLKFR	P0AAI3	P0A9Q5	203	84	27.94		
	SMVMTEAQKESTAYHEAGHAIIGR	KEKAIDALNMAAGFKK	P0AAI3	P76542	408	106	38.57	TLLAKAIAGEAK	HAIIKVKR	P0AAI3	Q47152	203	41	25		
	LVPEHDPVHKVTIIPR	GMLPKGPLGRAMFR	P0AAI3	P0AA10	433	111	37.72	GKEVMGKKENEMAQEK	TLLAKAIAGEAK	P37671	P0AAI3	1	203	24.34		
	TLLAKAIAGEAK	GAEKSKR	P0AAI3	P37645	203	380	36.66									
	SMVMTEAQKESTAYHEAGHAIIGR	MTQKNFVELRNVTKR	P0AAI3	P37009	408	0	36.52									
	DDDNKPLVYEPGLHFK	ETPQPDKTGMHILLK	P0ABC3	P0AGM0	44	8	35.22									
	AKQVALIAAAQDAETKAVELTVR	DDDNKPLVYEPGLHFK	P77306	P0ABC3	378	44	35.2									
	FVTKEKKDLIVDSYIK	AELDKAGKSR	P0ABC3	P0AF78	82	44	34.82									
	VDNVQILSAKPANMFEREVKNAMR	SVAKAK	P02929	P0AAI3	197	533	34.18									
	SMVMTEAQKESTAYHEAGHAIIGR	MIISEMQRK	P0AAI3	Q47688	408	0	34.08									
	LAEEIIYGPEHVSTGASNDIKVATNLAR	EELMAEAKK	P0AAI3	P0AAJ8	494	215	34.08									
	DLKGPQFLHIMTKKGR	LDVKDIVTDSR	P77488	P0ABC3	284	137	33.87									
	EQILKVHMR	QIEIKSGAK	P0AAI3	P0ABN5	332	220	32.47									
	AYKAQTILEAQGEVAR	MVQAKPCYVEDR	P0ABC7	Q46814	294	188	31.93									
XlinkX	EQILKVHMR	EELMAEAKK	P0AAI3	P0AAJ8	332	215	31.37									
	YMKTPTSATR	KNVGDLMQR	P0ABC3	P0AEF0	327	1	31.37									
	GVIGKYTMDR	MAEKQTAKR	P0ABC7	P0C093	198	0	31.37									
	DDDNKPLVYEPGLHFK	KEAAPAKAEKK	P0ABC3	P0A9Q7	44	876	30.57									
	IIDQEVKALIER	MSDFAK	P0AAI3	Q46890	550	0	30.1									
	LVPEHDPVHKVTIIPR	KVTLTKAGKR	P0AAI3	P52696	433	61	29.98									
	LDVKDIVTDSR	LKGAHIVMDK	P0ABC3	P0A749	137	152	29.98									
	SEIGRLDVKDIVTDSR	KGMAYITESR	P0ABC3	P32721	137	338	29.98									
	SMVMTEAQKESTAYHEAGHAIIGR	SQNDIIIRTHYKSPHR	P0AAI3	P76205	408	1	28.2									
	MKCKR	KFSDR	P0ADP9	P0ABC3	0	122	27.86									
	KIPHGDIRVAFTPDEEVGKGAK	LDVKDIVTDSR	P29745	P0ABC3	177	137	27.14									
	EQLDKYVANGGKR	EQILKVHMR	P42592	P0AAI3	546	332	26.6									
	DKVLDSALLSSANKRK	LDVKDIVTDSR	P71311	P0ABC3	3	137	26.05									
	TEFWLEKIGKNVER	SQGQEEAEKLR	P09184	P0ABC3	92	248	25.66									
	GVIGKYTMDR	MKCKR	P0ABC7	P0ADP9	198	0	24.47									
	FVTKEKKDLIVDSYIK	FNIDADKVNPR	P0ABC3	P0AFG8	85	881	23.96									
	FPEKGKTYSINEGNYIK	LVPEHDPVHKVTIIPR	P0A993	P0AAI3	199	433	23.24									
	SMVMTEAQKESTAYHEAGHAIIGR	GSLERIKQYQQVIDNYPK	P0AAI3	P39285	408	67	22.53									
	MSITAQSVYR	YMKTPTSATR	P21365	P0ABC3	0	327	21.51									
	FVTKEKKDLIVDSYIK	SDLAR	P0ABC3	P0AES4	84	1	20.78									
	DAGEKLWDAVTGQHDKDDQAKK	EQILKVHMR	P0ADE6	P0AAI3	13	332	20.11									
	KQKITDYLDEIYGGTFTATHLQK	SVAKAK	P76041	P0AAI3	1	533	17.08									
	SMVMTEAQKESTAYHEAGHAIIGR	EKLAQDRIKTK	P0AAI3	P26266	408	228	16.94									
	LVPEHDPVHKVTIIPR	SGSGKPNKDKVGKISR	P0AAI3	P0A7J7	433	97	15.89									