Proteome response of upper respiratory system following particle exposure

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LUND UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

Airborne particles can be emitted from both occupational and environmental sources especially during combustion, fume alteration processes and other dust forming tasks. Exposure to airborne particles has been associated with several diseases, e.g. cancer, cardiovascular disease as well as lung diseases such as asthma, bronchitis and COPD. Airborne particles from occupational and environmental sources contain a complex mixture of agents that can differ in physical, chemical and biological properties that may be closely related to the induced health effects. A major part of the underlying induced mechanisms causing the health effects are not fully understood and additional knowledge needs to be gathered by associating the different occupational and environmental airborne exposure sources with the induced biological response. The general aim of this thesis is to measure protein changes, with respect to both protein abundance and the absolute quantity of specific proteins in nasal lavage fluids following three different exposures; persulfate, welding fume particles and diesel exhaust. Changes in the protein composition of the upper airways could provide a better understanding of the underlying pathogenesis. The general aim was also to clarify the role of different particle parameters affecting the dose metric.

Protein-particle coronas were studied in an *in vitro* test carried out for two welding fume particle fractions; fine fraction (0.1- 2.5 µm) and ultrafine fraction (<0.1 µm) and two types of iron oxides; Fe_2O_3 (20-40 nm) and Fe_3O_4 (8 nm) at different particle mass concentrations by adding them to nasal lavage proteins. The proteins that bound to the different particles at different mass concentrations were further analyzed with two different mass spectrometry approaches; a targeted SRM LC-MS/MS and 2DE-MALDI-TOF-MS.

Proteomic analyses were performed on nasal lavage proteins sampled from hairdressers with and without bleaching powder associated rhinitis and an atopic group experimentally exposed to persulfate. Samples were collected before, 20 min, 2h and 5h after the persulfate challenge. The protein composition was determined with a targeted SRM method for 247 proteins.

In an exposure chamber, 11 welders with work-related symptoms in the lower airways were exposed to mild-steel welding fume particles (1 mg/m^3) and to filtered air, respectively. Nasal lavage samples were collected before, immediately after, and the day after exposure. The proteins in the nasal lavage were analyzed with two different mass spectrometry approaches, label-free discovery shotgun LC-MS/MS and a targeted, selected reaction monitoring (SRM) LC-MS/MS analyzing 130 proteins and four *in vivo* peptide degradation products.

In an exposure chamber, 19 healthy volunteers were exposed to diesel exhaust (300 µg/m^3) and to filtered air, respectively. Nasal lavage samples were collected before, immediately after, and the day after exposure. The proteins in the nasal lavage were analyzed with two different mass spectrometry approaches, label-free discovery shotgun LC-MS/MS and a targeted selected reaction monitoring (SRM) LC-MS/MS analyzing 144 proteins and two *in vivo* peptide degradation products.

Several proteins with biological relevance were altered after the respective exposures in the different study groups. This thesis suggests that the balance between proteases and antiproteases, disruption of the extracellular matrix, inflammation and immunosuppression are important induced effects by occupational and environmental particle exposure.It was also demonstrated that different particle parameters such as chemical composition, agglomerated particle size along with the primary particle size could determine the type of proteins that interact with them, and that such binding could cause an inhibitory effect of the bound protein, and cause an excessive effect on the downstream activity.

Key words: proteomics, mass spectrometry, upper airway response, nasal lavage, particles

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Cover image adapted from shutterstock.com illustration of airborne particles inhalation.

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To Mom and Dad

Contents

Populärvetenskaplig Sammanfattning

Luften i olika omgivningsmiljöer innehåller varierande halter av olika slags små luftburna partiklar som betraktas som föroreningar. Vi exponeras för nanopartiklar i den allmänna miljön utomhus såväl som inomhus och dessutom kan personal utsättas för partiklar på arbetsplatser, så kallad yrkesmässig exponering. Exponeringsnivåerna av partiklar kan skilja sig markant åt beroende på arbetsplats och yttre miljö. Studier visar att det finns ett samband mellan exponering för luftburna partiklar och ökad risk för sjukdom i luftvägar och hjärt, kärlsystem. Kunskapen är dock ofullständig för att kunna förklara vad det är som händer när olika slags partiklar når andningsvägarnas vävnader.

Ett sätt att studera detta är genom att analysera hur proteinutsöndring förändras hos människor som har exponerats för olika partiklar. Sådana studier kan ge information om hur partiklarna ger upphov till att starta eller förändra biologiska processer. Förutom att få en bättre förståelse för vad som händer i kroppen då partiklarna andas in kan proteinerna också användas som biomarkörer för att påvisa en exponering eller en hälsopåverkan.

Luftburna partiklar som vid inandning kan deponera någonstans i luftvägarna, beroende bland annat på form och storlek, kan i samband med det binda till proteiner som finns där och bilda ett proteinhölje på partiklarna. Ytterligare ett sätt att studera hur partiklar kan påverka viktiga processer i kroppen är att experimentellt studera vilka proteiner i biologiska matriser som binder in till olika partiklar. På så vis kan viktig information erhållas om sambandet mellan proteininbindning och påverkan på biologiska processer. Nässköljvätska kan fungera som en modellvätska då studier av partikel-proteininbindning mellan partiklar och protein i luftvägarna ska studeras.

Exempel på yrkeskategorier där det är vanligt med partikelexponering är frisörer och svetsare. Frisörer utsätts dagligen för en mängd kemikalier på arbetet. Många frisörer drabbas av luftvägsbesvär vilka ofta kan kopplas till hårblekningsmedel. Blekningsmedel innehåller bland annat en typ av ämne som kallas persulfater vilka tros vara en av huvudorsakerna till luftbesvären. Vid applicering av blekningsmedel i hår frisätts partiklar i luften, till stor del bestående av persulfater.

Svetsare utgör en arbetsgrupp som utsätts för betydande halter av partiklar som alstras i svetsprocessen. Ökade förekomster av besvär från luftvägarna har rapporterats i många studier. Vad som utlöser besvären och mekanismen bakom de svetsrök relaterade symptomen är ofullständig.

Dieselpartiklar från motorfordon är en starkt bidragande orsak till luftföroreningar, och man har sett i befolkningsstudier att exponering för luftföroreningar kan orsaka en rad olika skador så som hjärt-kärl och lungsjukdomar.

Under kontrollerade former exponerades försökspersoner för olika typer av partiklar varpå effekter har studerats genom att kartlägga förändringar i proteinmönstret i nässköljvätska.

Resultaten visade att flera proteiner involverade i olika reaktioner i immunförsvaret förändrades vid exponering för persulfat, svets eller dieselpartiklar. Flera av dessa proteiner var proteaser/antiproteaser, extracellulära strukturproteiner samt inflammation/ inflammationshämmande proteiner, och att balansen mellan dessa proteiner är en viktig aspekt att ta hänsyn till vid partikel exponering. Det visades även att olika partikelparametrar såsom kemisk sammansättning, agglomererad partikelstorlek tillsammans med primärpartikelstorleken kunde bestämma vilka proteiner som band in till de olika partiklarna och att en sådan bindning kan påverka protein funktionen.

List of papers

This thesis is based upon the following papers, referred to in the text by their Roman numbers (I-IV). Published papers are reproduced with the permission of the publishers.

I. Ali, Neserin; Mattsson, Karin; Rissler, Jenny; Karlsson, Helen M; Svensson, Christian R; Gudmundsson, Anders; Lindh, Christian H; Jönsson, Bo AG; Cedervall, Tommy; Kåredal, Monica. "Analyses of Nanoparticle-Protein Corona formed *in vitro* between Nanosized Welding particles and Nasal lavage fluid" *Nanotoxicology*. **2016**; 10 (2): 226-234.

II. Mörtstedt, Harriet; Ali, Neserin; Kåredal, Monica; Jacobsson, Helene; Rietz, Emelie; Kronholm Diab, Kerstin; Nielsen, Jörn; Jönsson, Bo; Lindh, Christian, "Targeted proteomic analyses of nasal lavage fluid in persulfate challenged hairdressers with bleaching powder associated rhinitis" *Journal of Proteome Research*. **2015**; 14 (2): 860–873

III. Ali, Neserin; Ljunggren, Stefan; Karlsson, Helen M; Wierzbicka, Aneta; Pagels, Joakim; Isaxon, Christina; Gudmundsson, Anders; Rissler, Jenny; Nielsen, Jörn; Lindh, Christian H; Kåredal, Monica. "Comprehensive proteome analyses of nasal lavage samples after controlled exposure to welding nanoparticles shows an induced acute phase and a nuclear receptor, LXR/RXR, activation that influence the status of the extracellular matrix" (*Submitted to Clinical Proteomics, 2017-07-26*)

IV. Ali, Neserin; El-Hams, Maha; Ljunggren, Stefan; Nielsen, Jörn; Wierzbicka, Aneta; Gudmundsson, Anders; Rissler, Jenny; Albin, Maria; Lindh, Christian H; Karlsson, Helen M; Kåredal, Monica. "Proteomic analyses of nasal lavage fluids collected from healthy volunteers experimentally exposed to diesel exhaust revealed activated lipid metabolism and inflammatory responses" (*Manuscript 2017*)

Abbreviations

A1AT	Alpha- 1-antitrypsin
A2MG	Alpha- 2- macroglobulin
AC	Accession number
COPD	Chronic obstructive pulmonary disease
DTT	Dithiothreitol
ECM	Extracellular matrix
FDR	False discovery rate
FEV	Forced expiratory volume
FVC	Forced vital capacity
F_{WF}	Fine welding fume particle fraction
GMAW	Gas metal arc welding
IL	Interleukin
IPA	Ingenuity Pathway Analysis
LC	Liquid chromatography
LMM	Linear mixed model
LOD	Limit of detection
LXR/RXR	Liver X receptor/Retinoid X receptors
MMP	Matrix metalloproteinase
MS	Mass Spectrometry
MS/MS	Tandem mass spectrometry
NLF/NL	Nasal lavage fluid/ Nasal lavage
PM	Particular matter
SRM	Selected Reaction Monitoring
TOF	Time of flight
U_{WF}	Ultrafine welding fume particle fraction

Introduction

General background

Airborne particles can be emitted from both occupational and environmental sources especially during combustion, fume generating processes and other dust forming tasks [1-4]. Exposure to airborne particles has been associated with various diseases e.g. cancer, cardiovascular diseases and lung diseases such as asthma, bronchitis and COPD [5-7]. Airborne particles from occupational and environmental sources contain a complex mix of agents that can differ in physical, chemical and biological properties that may be closely related to the induced health effects [8]. Depending on the properties of the particles, they can be inhaled and deposited at different regions of the respiratory tract [9]. Studies have shown that some nano-sized particles can be more toxic compared to the larger sized bulk material [10]. For smaller particles, the total surface area increases compared to larger particles at the same mass unit, making them more available for interaction with biological matrices. The potential health risk caused by the particle exposure depends on the magnitude and nature of the exposure source.

Although many of the respirable particles emitted from occupational and environmental sources have been associated with different physiological and clinical responses, knowledge about parts of the pathogenesis is still lacking. This makes the identification and quantification of biological responses associated with different particle exposures highly relevant to investigate in order to explore the underlying mechanisms. Proteomic analyses of biological samples can help gaining such information.

Occupational and environmental exposures

There are a number of occupational and environmental sources of emitting particles in the respirable range. In this thesis, the biological effects following exposure to particles generated during hair bleaching, welding and diesel combustion have been studied.

Exposure to persulfate

Hairdressers are exposed to a wide diversity of chemicals in products such as hair dyes, bleaching products, permanent wave solutions, semi-permanent hair colors, hair sprays and various styling products that can potentially damage the respiratory system [11]. When using the products the hairdresser get exposed to emitted particles as well as gaseous compounds which can be irritating to the epithelium of the airways [12]. Hair bleaching is the activity that gives rise to the most prominent and frequent respiratory symptoms among hairdressers [13, 14]. Bleaching powder mainly contains persulfate salts, which can act as allergens and airway irritants. In Sweden, there is no threshold limit value for persulfates exclusively, but the American Conference of Governmental Industrial Hygienists (ACGIH) has concluded that 0.1 mg/m^3 is a threshold limit value for persulfate with an eight hour time weighted average [15]. The hair bleaching products can be in the form of powder, granules or gel, and contain persulfates in concentrations up to 60%. Mixtures with an oxidizing agent, typically hydrogen peroxide, are made prior to the application into the hair and during this preparation and application particles typically less than 10 µm are emitted [16] into the air and are easily inhaled and deposited in the respiratory tract. This makes persulfate of extra interest to study. In this thesis, the biological response from the upper airways following persulfate exposure was studied in three different groups in **paper II**.

Exposure to welding fume particles

It has been shown that although the exposure levels for welders do not normally exceed current Swedish permissible occupational exposure limits for inorganic respiratory dust (5 mg/m^3), there is a high frequency of upper and lower respiratory symptoms among welders in Sweden [17, 18]. During welding, base materials (usually metals) and a filler material are fused at high temperatures. During this process, fumes are generated that contains a complex mixture of agglomerated metallic particles (a network of interacting particles, typically ~100–1000 nm) and gases. The agglomerates are built up of primary nanoparticles, which can range between 2-70 nm in diameter [19]. Particles in the submicron range can easily be inhaled and deposited in the respiratory tract. Depending on the welding technique and electrode used, the fumes can contain different types of metallic particles with different sizes and morphologies. The most common generated metals are iron, manganese, copper, chromium and zinc and oxides of those and the most common gases are carbon monoxide, hydrogen fluoride, nitrogen oxide and ozone. The nanosized particles may have different physical and chemical properties compared to larger sized particles composed of the same material [10, 20]. Studies have also shown that smaller particles may induce a

higher toxicity compared to bulk material [21]. However, no consensus regarding dose metrics has been reached so far. The different biological characteristics may be associated with the difference in surface area to mass ratio. This ratio is for nanoparticles high, making them more available for interaction with biological systems. Thus, comparing different particle sizes and chemical composition of welding fume particle fraction were studied in **paper I.**

Welding can be conducted using different welding methods, each associated with different health and safety risks [22-24]. Thus, the potential health risk may depend both on the nature and the magnitude of the exposure source. Welding in mild steel accounts for the majority of all welding. The most common method is gas metal arc welding (GMAW) [25]. This fact makes welding fume particles generated from mild steel and GMAW of extra interest to study. The biological response from the upper airways induced by welding fume particles was studied in a group of welders with lower respiratory symptoms in **paper III**.

Exposure to diesel exhaust

Motor vehicle emissions constitute a major source of air pollution [26] and diesel fuel combustion is a large contributor to the particular matter (PM). Diesel exhaust is produced by the combustion (burning) of diesel fuel. The exhaust consists of a complex mixture of gases and soot particles, consisting primarily of solid elemental carbon cores, traces of metallic compounds and organic material like PAHs. The particles are predominantly less than 0.1 μm and gases consisting of carbon monoxide, carbon dioxide, oxides of nitrogen (e.g., nitrogen oxide, nitrogen dioxide) and oxides of sulfur (e.g., sulfur dioxide) [27]. The exact composition of the exhaust depends on a number of factors including the type of engine, how well maintained the engine is, type of fuel, speed and load on the engine and emission control systems. In this thesis, the biological response from the upper airway following a well-defined chamber exposure of diesel exhaust was studied in healthy volunteers in **paper IV**.

Health effects

Hairdressers often experience occupational associated symptoms [12, 14]. Case studies of hairdressers have described nasal symptoms, mostly blocked noses and dry coughs, but some studies have also found that hairdressers frequently have asthma [13], rhinitis [28] and other respiratory diseases. It has been described that many hairdressers leave the occupation and it has been suggested that the high dropout rate may be linked to their experience of symptoms [29, 30].

Welders have been described to experience a number of negative health effects and symptoms including airway irritation [24, 31], asthma [32] and susceptibility to pulmonary infection [33-36], "metal fume fever", chronic effects including central nervous system problems [37, 38], kidney damage and emphysema siderosis (a benign form of lung disease caused by particles deposited in the lungs) [17, 39], dry throat, stuffy nose [17], sinus problems [40, 41], chest pain and breathing difficulty that tends to clear up when exposure stops. Welding was just recently classified as carcinogenic to humans by IARC.

Diesel exhaust exposures have been associated with health problems, cardiovascular disease and lung diseases [42] such as asthma [43] and COPD [44, 45]. Exposure to diesel exhaust can cause lung irritation causing coughing [46], wheezing [47] and difficult breathing, itchy or burning eyes and nasal irritation [48-50]. Years of exposure to diesel exhaust may increase the risk of lung cancer and possibly bladder cancer [51].

Biological response and mechanisms

Several underlying mechanisms have been suggested to explain the symptoms induced by persulfate exposure. Studies show that there is an inconsistent association between IgE responses and persulfate exposures [28, 52-54]. Instead, a nonspecific hypersensitivity may explain the bleaching powder associated symptoms. Furthermore, Th1 signaling and oxidative stress may be important underlying mechanisms [54]. Additionally, different biomarkers were upregulated in symptomatic hairdressers compared to asymptomatic hairdressers [55], suggesting different underlying mechanisms triggered in different groups after persulfate exposure. Further studies are needed to clarify the mechanism for persulfate-associated nasal symptoms. In this thesis, the effect on protein level induced by persulfate exposure was studied in three different groups (hair dressers with and without beaching powder associated rhinitis and an atopic group without work related beaching powder exposure) in **paper II** with a proteomic screening method.

Several studies have investigated how the pulmonary inflammation and pulmonary function was affected by diesel exhaust exposure or by welding fume exposure by analyses of specific inflammatory biomarkers [25, 27, 56, 57]. The underlying mechanisms causing the pulmonary symptoms are still not fully understood, although it has been suggested that inflammation and oxidative stress are important underlying mechanisms inducing health effects following diesel exhaust exposure [27] and welding fume exposure [22, 35, 58]. However, there is a need to explore the possibility of yet other mechanisms. Proteomic analyses may provide such information. The induced effect on protein levels induced by welding fume

particle exposure as well as diesel exhaust exposure was investigated in **paper III** and **IV**.

Upper airway proteome

Biological samples can be obtained from humans to assess the induced biological effect due to an external exposure [59, 60]. It is important to consider if the markers measured in the biological samples reflect the induced processes of the target organ. If an association between the exposure and the induced effect can be established, then this constitutes a biomarker of effect.

For the respiratory system, samples such as exhaled air, sputum, nasal lavage fluid (NLF), and bronchoalveolar lavage fluid (BALF) could be obtained, containing markers that can indicate or show local biological change [61]. The upper respiratory system is the first line of defense against foreign microbe or particulates compounds that are inhaled through nasal breathing. The nasal cavity is lined and coated with a pseudostratified columnar ciliated epithelium (**figure 1**). All cells are attached to the basal membrane. Basal cells lie on the membrane and show no contact with the epithelial surface. Their specific morphologic features are desmosomes for cell adhesion. The epithelial cells are ciliated cells which handle mucociliary clearance by trapping particles in the mucus layer which are moved upwards. Nasal secretions contain a variety of proteins, mucus, serous fluids, and secretions from epithelial and immunological cells such as goblet cells, submucosal glands and immunological cells. Secretions also contain transudate from plasma containing, e.g. proteases, immunological antibodies, anti-proteases, structural proteins and transport proteins [62]. Nasal lavage fluid could therefore be a suitable biological sample to explore the induced biological effect in. Protein changes in nasal lavage were studied in **paper I-IV**. The protein pattern in nasal lavage fluid match to a great degree the proteins in bronchial lavage fluid, it might also serve as a proxy for lower airway response [63-66].

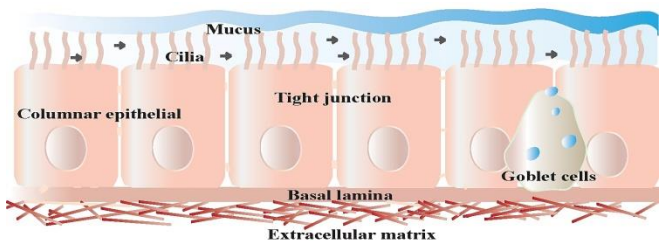

Figure 1.
Nasal mucousa. (Picture by Neserin Ali)

Proteomics

The proteome consists of all proteins expressed in an organism at a given time point. By studying the proteome with analytical techniques, so called proteomics, changes of the protein composition can be identified that can explain underlying pathogenesis induced by various external exposures. The complexity of biological samples can limit the number of identifications and quantification of proteins with biological relevance of an induced effect. In plasma for example, high-abundance proteins such as albumin and transferrin constitute approximately of 99% of the total protein, the remaining 1% is assumed to include many proteins that are typical of low abundance which can be of potential biomarkers [67]. The wide variation in types of mass spectrometry techniques regarding instrumentation, fragmentation and analysis strategy have made the identification and quantification of the proteome with mass spectrometry into an indispensable tool for proteomics research. A combination of different techniques improves the likelihood of detecting important protein changes that could be lost due to limitations of specific mass spectrometry difficulties [68].

Discovery shotgun applies a technique by which all possible peptides can be detected, which generates a global protein profile based on the spectral information forming the basis for peptide sequencing and identification. Although the shotgun approach is conceptually simple, it results in greatly increased complexity of the generated peptide mixture, requiring highly sensitive and efficient separation. Not all peptides resulting from the digestion of a protein can be observed or correctly identified with MS analyses, especially those with diverse or unexpected modifications. Furthermore, the limited dynamic range of mass spectrometric analyses only allows for the peptides present at high relative abundance to be preferentially sampled, if no additional depletion or fractionation steps are added, with the addition of the lack of valid quantitative information especially when using label-free quantification and with relatively large numbers of missing values.

Targeted proteomics with liquid chromatography (LC)-coupled selected reaction monitoring (LC−SRM) measure only predetermined peptides. This approach offers a better opportunity to validate multiple biomarker candidates simultaneously and in a more high-throughput fashion [69, 70]. But it lacks the advantage of identifying new proteins. It has been proposed to combine tandem LC−MS/MS discovery shotgun with complementary validation techniques. A comparison between discovery and targeted analyses shows that discovery proteomics offers high data density while targeted offers selectivity, a broad dynamic range, and a high degree of reproducibility and repeatability (**figure 2**) [68].

With techniques that enable high throughput profiling, identification of a subset of proteins with changed levels associated with an exposure can be determined. Interpretation of each protein individually can be time-consuming and it might fail to provide biological meaning. Instead, pathway analyses can be applied to help explain how the identified proteins are connected. Pathway analyses identifies common signaling molecules shared between the proteins and if the several proteins can be identified in the same pathway, then there is a higher likelihood that this pathway is involved in the biological response [71, 72]. Pathway analyses were therefore applied in all **papers I-IV**.

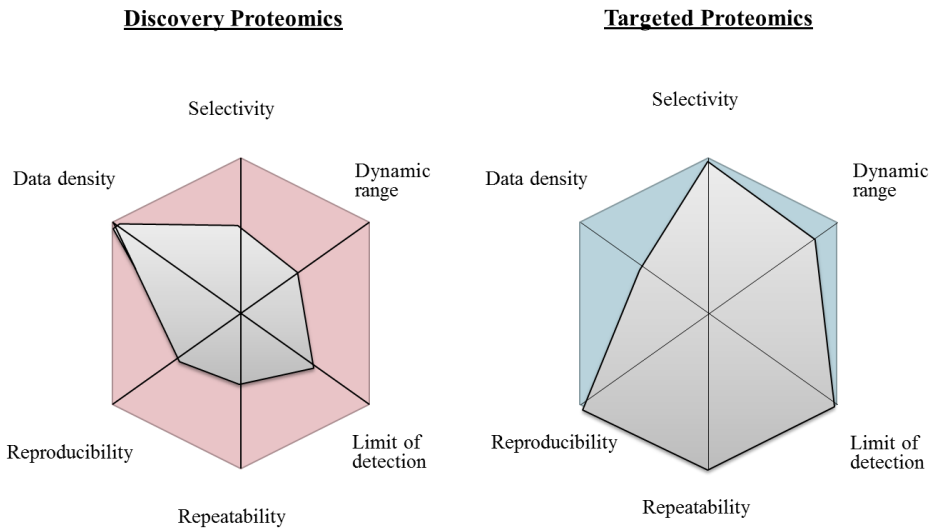

Figure 2.
Comparison between discovery and targeted proteomics. (Modefied picture by Neserin Ali)

Biomarkers

Biological markers (biomarkers) can be used as indicators of an induced or changed biological state or the presence of a disease [59]. Ideally, a biomarker should be collected in a non-invasive way, it should be readily available, have a high sensitivity, high specificity and known biological half-life, providing diagnostic or prognostic information to the clinician. Commonly only a few biomarkers are used to assess an effect, whereas due to redundant function of proteins, it could be necessary to measure a combination of a panel of proteins/biomarkers in order to associate them with a specific exposure or effect [73-75]. In this thesis, the studies were based on combinations of different mass spectrometric methods to gain as comprehensive protein data as possible.

Aims

General aims:

- To study protein-particle interaction between occupationally formed particles and proteins in the upper airways.
- To study effects on the protein level in the respiratory system as result of occupational and environmental particle exposure.
- To elucidate the mechanisms connected to the altered protein levels.

Specific aims:

- To identify proteins in nasal lavage fluid that binds to the welding particles.
- To clarify the role of different particle parameters in the protein binding (particle size and chemical composition).
- To explore if particle binding to proteins can alter the original function of the protein.
- To explore the biological effects related to persulfate exposure on the protein level.
- To explore the biological effects related to welding fume particle exposure on the protein level.
- To explore the biological effects related to diesel exhaust exposure on the protein level.

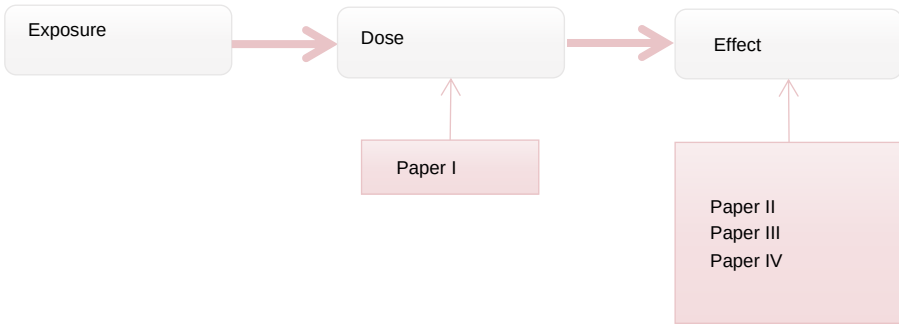

Figure 3.
Overview of the papers included in the thesis

Materials and Methods

Study design

In this thesis, one *in vitro* experiment and three controlled human exposures were studied.

Protein corona study

The protein-particle corona formed when welding fume particles were added to nasal lavage proteins were studied in an *in vitro* experiment in **paper I.** Two welding fume particle fractions F_{WF} (0.1- 2.5 μm) and UF_{WF} (<0.1 μm) and two types of iron oxides Fe_2O_3 (20-40 nm) and Fe_3O_4 (8 nm) particles were suspended in water. The agglomerated mean hydrodynamic particle size changed to 130 nm for F_{WF}, 99 nm for UF_{WF}, 100 nm for Fe_2O_3, and 26 nm Fe_3O_4. Three different particle mass concentrations (400, 200 and 100 μg particles/ml) were studied for each particle type. Each particle type and mass concentration was added to nasal lavage protein (800 μg /mL) separately, and the preparations were incubated for 6h. Proteins bound to the particles (the protein corona) were separated from unbound proteins by centrifugation. The proteins bound to the different particles at the different mass concentration were further analyzed with two different mass spectrometry approaches, a targeted SRM LC-MS/MS and 2DE- MALDI-TOF-MS.

Experimental challenge with persulfates

In **paper II**, hairdressers with (s, n = 15) and without (ws, n = 14) bleaching powder-associated rhinitis and atopic volunteers (a, n = 12) with no prior work related exposure to persulfate were challenged with persulfate. All groups consisted of female volunteers. Work-related symptoms were defined as those worsened at the workplace and/or recovery during weekends or holidays away from the workplace. None of the hairdressers had a history of atopy or asthma and the atopy by history group was not defined of having asthma. The nasal challenge was performed by spraying 0.001% fresh solution of potassium persulfate in

isotonic saline solution, and after 20 min with a 0.01% solution (w/v) in the nasal cavity of the study subjects. A total of 300 μg of each solution was sprayed into the nasal cavities in turns. To evaluate if the nasal lavage procedure itself induced any protein changes, nasal lavage fluid was collected from six subjects according to the same protocol and time schedule as used in this study but without the persulfate challenge.

Chamber exposure

In **paper III** and **paper IV,** the subjects were exposed to either welding fume particles or diesel exhaust in an exposure chamber (22 m^3) which facilitated an inhalation study of a controlled environment surrounding the study subjects. A well characterized exposure concentration was provided. The volunteers were exposed to filtered air (blank exposure) and to the real exposure (welding fume particle or diesel exhaust). The acute response was studied following the exposure in **paper III** and **IV.**

Welding fume particles

In **paper III** 11 male non-smoking welders, ranging from 29 to 66 years of age, with work related lower airway symptoms (wheezing, dyspnoea, and/or coughing) the last month were studied. A medical examination was performed before the exposure day, examining for any upper airway symptoms, performing a methacholine test and checking the lung function (FEV_1% and FVC% of predicted) of the welders. A physical examination was performed including rhinoscopy to exclude any nasal conditions that may mimic or generate rhinitis-like symptoms. The exposures were performed on two separate Mondays, each for 5.5h exposing the welders to $PM_{2.5}$ ~1000 μg /m^3 welding fume particles or filtered air. The welding fume particles were generated by gas- metal arc welding in mild steel and collected in a closed chamber. Gases emitted from the welding were removed and only the particle fraction was further fed into the exposure chamber. The composition of the welding fume particles was mainly iron oxides and up to 20% manganese. The primary particle size ranged from 2 mm to 70 nm, and aggregates with a mean mobility diameter of 160 nm were formed.

Diesel exhaust

In **paper IV**, 18 healthy non-smoking volunteers, nine male and nine female, ranging from 40-66 years of age (mean 51 years) were included in the study. The subjects included had a negative skin prick test and exhibited no physical signs of asthma or any other respiratory symptom. The exposures were performed on two separate weeks each for 3h exposing the volunteers to PM_1~300 μg/m^3 diesel exhaust or filtered air. The diesel exhaust was generated from a passenger car

(Volkswagen Passat TDI, -98, 1900 cm^3, 81 kW) when idling. The fuel used was Swedish Environmental Class 1 diesel with sulfur content of less than 10 ppm, aromatics 4% volume and PAHs less than 0.02% volume.

Nasal lavage sampling

Nasal lavage samples were collected from volunteers in **paper I** and pooled together before analysis. In **paper II-IV** the nasal lavage was collected from the different subjects in the different exposures both before and after exposure. Nasal lavage was collected from the volunteers in **paper II** by instilling 15 mL of isotonic saline solution in the nasal cavity. This procedure was repeated three times in the left and the right nostril alternately resulting in approximately 45 mL nasal lavage at each sampling time for each subject. The first sample was a washout (NL 0, not analyzed), and the second lavage before the challenge was used as the baseline (NL 1), the third sample was collected 20 min after the persulfate challenge (NL 2), and the fourth was taken 2h after the persulfate challenge (NL 3), and the last one was taken 5h after the persulfate challenge (NL 4). The samples were stored at -80°C until analyses.

Nasal lavage samples were collected from volunteers in **paper III** and **IV** by instilling the nasal cavity with 18 ml of isotonic saline solution. The subjects were first sampled with a washout (NL 0, not analyzed) and then the second lavage before the exposure was used as the baseline (NL 1) for the post-challenge samples. The third sample was collected immediately at the end of the exposure (NL 2), and the fourth was taken at 18–20h after the end of the exposure (NL 3). All samples were stored at -80°C until analyses. The total protein content in each nasal lavage fluid sample was determined using a BCA protein assay kit.

Sample Preparation

Protein corona

In **paper I**, the nasal lavage samples were concentrated and desalted. Particles (F_{WF}, UF_{WF}, Fe_2O_3 and Fe_3O_4) were suspended in water and sonicated to obtain a homogenous solution. Three different particle mass concentrations (400, 200 and 100 μg particles/ml) were studied for each particle type. Each particle type and mass concentration was added to the nasal lavage protein (800 μg /mL) separately and the preparations were incubated for 6h. Then the samples were either reduced,

alkylated, and trypsin digested on the protein corona, prior to the analyses with LC-MS/MS or the proteins on the protein –corona were denatured with a urea, prior to 2-DE separation (**figure 4**). The amount of proteins loaded on the 2-DE gel was 50 µg of the bound proteins.

Pooled samples in human exposure studies

Individual nasal lavage samples were pooled in **paper III** and **IV.** The nasal lavage was evaporated and then reconstructed in 50 mM ammonium acetate. Equal amounts of total protein (50 µg) were pooled from all subjects with samples collected at all time points (n = 9), resulting in six pooled samples for each group studied in **paper III** and **IV**. The nasal lavage proteins were further reduced, alkylated and then desalted with centrifugal filters (cut-off 3 kDa) (**figure 4**). Prior to shotgun analyses the samples were trypsin digested.

Individual samples in human exposure studies

Preparations of individual samples were also performed. The samples were evaporated and dissolved in 50 mM ammonium acetate to a concentration of 4600 µg/mL in **paper II** and 400 µg/mL in **paper III** and **IV**. Each individual sample was desalted, reduced, alkylated, and then trypsin digested in **paper II** (**figure 4**). The individual samples in **paper III** and **IV** were reduced, alkylated, trypsin digested, spiked with isotopically labeled standards from matrix metalloproteinase (MMP)9 (4 fmol/µL), alpha-1-antitrypsin (A1AT) (4 fmol/µL), alpha-2-macroglobulin (A2MG) (4 fmol/µL), and desalted on a SPE column.

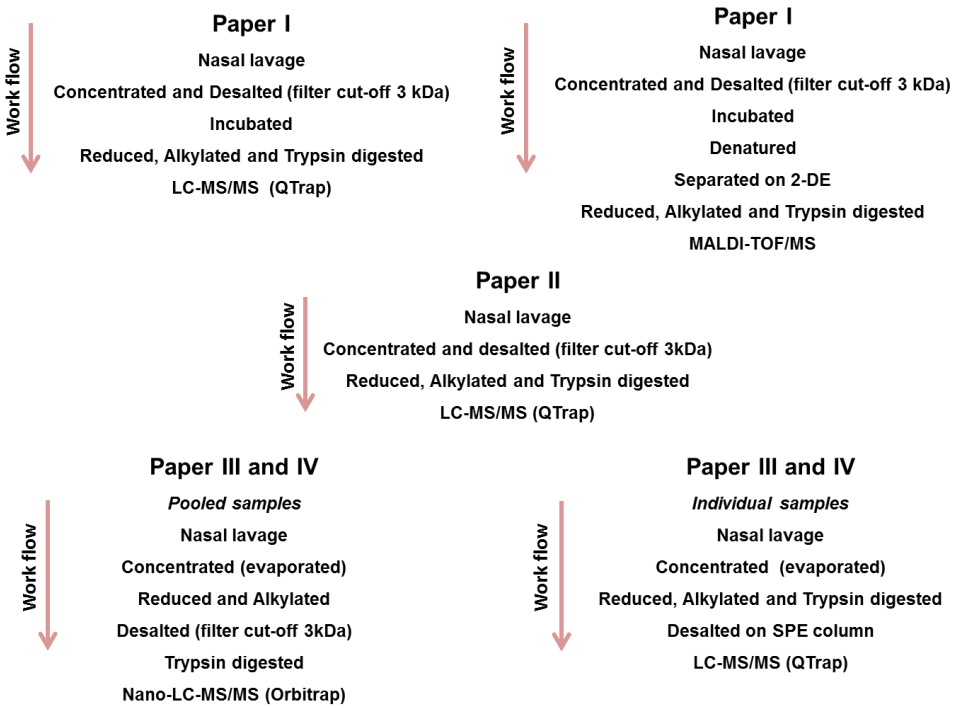

Figure 4. Sample preparation work flow in paper I-IV
The sample preparation workflows were adjusted for each study design. Desalting after sample preparation reduces the DTT and iodoacetamide in the final solution before analyzing with LC-MS. Such a workflow had a less negative effect on the LC-MS system, such as clogging and losing the intensity, better persistence on the column and intensity in the MS system. Internal standards would have been lost on a filter of 3 kDa cut-off when introduced to the samples in **paper III** and **IV**. Introducing an internal standard in **paper III** and **IV** made it necessary to desalt on a SPE column on peptide level.

Proteomic analyses

MS-platforms

Different combinations of three types of mass spectrometry in combination with three different separation techniques were applied in this thesis. In **paper I**, a two-dimensional gel electrophoresis (2-DE) analysis followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS; voyager-de pro, Applied Biosystems) was applied. In **paper I-IV**, an online micro liquid chromatography technique combined with a hybrid triple quadrupole/linear ion trap mass spectrometer (UFLCXR; Shimadzu corporation) equipped with a

turbo ion spray source (5500QTRAP, Applied Biosystems) was applied. In **paper III** and **IV**, an online nano-separation liquid chromatography technique (EASYnLC, Thermo scientific) coupled to a high-resolution mass spectrometer (Orbitrap Velos Pro, Thermo Fisher) was applied.

Discovery proteomics

Denatured proteins from the protein corona were analyzed with 2-DE combined with a MALDI-TOF-MS

In **paper I**, the spectra were processed with data explorer (version 4.0, Applied Biosystems, foster city, ca) for protein identification. The mass list (mass+H+) generated from the 40 most abundant peaks of the MALDI a spectrum was submitted to a database search (NCBI or Swiss-prot). In **paper III** and **IV** protein digest from pooled nasal lavage samples were analyzed with a nano liquid chromatography system with a reversed phase column coupled to a high resolution mass spectrometry (Orbitrap Velos Pro). Protein identification and relative quantification were performed with MAXQuant software and a human database downloaded from UniProt.

Relative quantification

In **paper I**, the 2-DE proteins were visualized using a cooled charged-coupled device camera digitizing at 1340 × 1040 pixels resolution (Fluor-S Multi-Imager, Bio-Rad) in combination with a computerized imaging 12-bit system designed for evaluation of 2-DE patterns (PDQuest version 7.1.1, Bio-Rad). The intensity of the protein spots on the 2-DE were used to evaluate the protein abundance in the protein coronas of the different particles. The percentage abundance of each protein normalized to the total intensity of each 2-DE of each particle. In **paper III** and **IV**, the protein levels were determined using label free quantification (LFQ) based on the peptide intensity obtained from the Orbitrap runs. Relative protein quantification was performed by normalizing each protein level against the protein level found in the baseline sample (the nasal lavage sample collected before exposure) for each exposure. Thus, the ratios were NL2/NL1 and NL3/NL1 respectively. For a protein to be further evaluated with the targeted method, it had to be detected in at least 50% of the samples and have a ratio for being categorized as an increased or decreased protein level of >1.3 (in **paper III**), >1.2 (in **paper IV**) or <0.8, respectively.

Targeted proteomics

In **paper I** and **II**, a comprehensive SRM method (previously developed) was used to relatively quantify nasal lavage proteins. 245 nasal lavage proteins were targeted in **paper I**, and in **paper II** 247 nasal lavage proteins and five oxidized peptides [76]. In **paper III**, 130 proteins and four *in vivo* peptide degradation products were relatively quantified, and three proteins were absolutely quantified. In **paper IV**, 144 proteins and two *in vivo* peptide degradation products were relatively quantified and three proteins were absolutely quantified. In total 71 proteins were targeted in all papers (**table 1**).

Table 1. The number of targeted proteins in common in paper I-IV.
In total 71 proteins were targeted in all papers.

	paper I	paper II	paper III	paper IV
paper I	-	245	73	73
paper II	245	-	76	91
paper III	73	76	-	102
paper IV	73	91	102	-

Relative quantification

Label-free peptide quantification was performed in all four **papers I-IV** by extracting peptide signal intensities. The ion signal intensity approach uses the extracted chromatographic area to compare peptide abundances across samples.

Absolute quantification

The concentration of MMP9, A1AT and A2MG proteins were measured in **paper II** and **IV** using a synthetic stable isotope-labeled peptide at a known concentration combined with a synthetic peptide added at different concentrations generating a calibration curve. The choice of the peptide was based upon previous sampling results.

Normalization

Normalization of the data was applied in **paper II- IV**, which accounts for variations in sample handling and instrument operation. In **paper II**, normalization was applied to the data by dividing each protein fold change with a correction factor. The median of all protein fold changes for a subject and for each time-point was calculated and used as the correction factor. This implies that for each subject, there were three correction factors, one for each time-point. This normalization was based on the assumption that the majority of the proteins do not change in

abundance. Further, for **paper III** and **IV**, the normalizations were applied by using the isotopically labeled peptides from MMP9, A1AT and A2MG (two precursor ions were used for this peptide, each of these two was separately used as a global normalizer)

Statistical analyses

For the majority of proteins, more than one peptide was measured. Mean total peak areas of duplicate analyses were calculated. In **paper I** and **II**, the protein ratios were calculated as the median of peptide ratios. In **paper I**, the peptide ratio was determined as the total peak area of the peptide bound divided by the total peak area of the peptide unbound. In **paper II**, the peptide ratio was determined by relating the mean total peak area for each time point to the baseline sample. The oxidation degree of the oxidized peptide in **paper II** was estimated as the ratio between the total peak area of the oxidized and the corresponding unmodified peptide. To assess the changes of the proteome level in **paper III** and **IV**, the statistical analyses were conducted on the normalized peptide levels.

Linear mixed model

A linear mixed model (LMM) was used in **paper II-IV** allowing each subject to serve as its own control. Depending on the study design different variables were included in the statistical analyses, such as the time of sampling, exposure, and study group. This improves the precision of the experiment by reducing the size of the error variance, but additional assumptions concerning the structure of the error variance must be made. Furthermore, mixed models allow us to make greater use of incomplete data, such as for individuals that had missing data. The significant changes were represented by the estimated marginal means for each group and time point differing from ratio 1 in **paper II**. The significant change was represented by the estimated marginal mean effect between the exposures in **paper III** and **IV** and the blank exposure. The mean value of the estimated marginal mean was calculated from several significantly changed peptide representing the same protein in **paper III** and **IV**.

Non-parametric methods

In **paper I**, the size differences between F_{WF} and UF_{WF} were statistically evaluated using the Mann–Whitney test. The Jonckheere–terpstra trend test was used for

proteins that showed a decreased $R_{b/u}$ trend with decreasing particle concentrations, and p-values ≤ 0.05 were considered significant. In **paper II**, differences in oxidation degree between the groups at each time point (baseline, 20 min, 2h, and 5h) were analyzed using the Kruskal−Wallis H test. Differences between the baseline time point and the other time points (20 min, 2h, and 5h) in oxidation degree were analyzed using the Friedman and the Wilcoxon signed-rank tests. The differences between the time points were analyzed for each group separately and also for all subjects regardless of group. Wilcoxon signed rank test was also used in **paper III** and **IV** to measure differences between the different exposure groups at the different time points. Spearman's rank correlation was also used in **paper III** to examine the associations between MMP9 concentration and FEV_1 % and FVC%.

Data evaluation

IPA pathways analyses were used in all four **papers I-IV**, to identify the biological relevance of the differentially changed proteins. The results were summarized based on the known pathways, diseases, functions and connecting regulators connected to the significantly changed proteins. Pathway analysis is based on current knowledge about different proteins and their involvement in different interactions and pathways [77]. The input data are introduced by a cut-off of the changed proteins, or significantly changed proteins. The pathway analysis summarize complex biological processes in a comprehensive way, however, these summaries may omit important details by grouping entities, leaving out alternative routes, and imposing artificial boundaries [78, 79]. Reality is much more complex than what is depicted in a typical canonical pathway. Therefore, this was just used to help to summarize the biological relevance of the induced protein changes by the different exposures.

Results and comments

Protein-particle interaction

Protein identification

In **paper I**, the experiments showed that different particle sizes and chemical compositions generated an overall different protein composition of the corona. Approximately equal amounts of proteins were detected to have a high affinity with the different particles; 15 of 245 targeted proteins interacted with F_{WF} with a high affinity, 17 proteins interacted with UF_{WF} with high affinity, 20 proteins showed high affinity to Fe_2O_3 and 20 proteins showed high affinity for Fe_3O_4. The protein corona of the smallest particles, Fe_3O_4 was distinct from the coronas of the three other particles. Some nasal lavage proteins bound to the particles to a large degree. Antileukoproteinase bound to a large degree to UF_{WF} and Fe_2O_3 particles, but only a smaller fraction of this protein bound to F_{WF} and Fe_3O_4.

Protein functionality

If the binding affects the function of the protein it might have clinically relevant implications. Antileukorproteinase is a highly abundant protein in nasal lavage fluid and it has anti-protease functionality. Therefore, it was selected for further functionality testing due to the high abundancy in the nasal lavage and high ratio bound to the particles.

A loss of inhibitory function of antileukoproteinase was observed when the protein was incubated with UF_{WF} and Fe_2O_3 particles in an ELISA assay. The F_{WF} particles, however, appeared actually to not cause any loss of the inhibitory function of antileukoproteinase. The chemical composition differed between the UF_{WF} and Fe_2O_3 particles. Additionally, the chemical analyses revealed that the two welding fume fractions of F_{WF} and UF_{WF} did not differ in chemical composition. Thus, the particle size or the aggregated form difference between the two welding fume fractions was the factor that induced this type of results. Therefore, it is difficult to predict the potential influence that particle binding has

on protein functionality, but this is still a relevant factor to investigate. The results obtained for protein functionality were dependent on the particle size and chemical composition. This suggests that the binding of proteins to particles may be an important factor in a toxicological response due to any functional alteration induced during the particle-protein interaction.

Binding determining parameters

Plotting the total nasal lavage proteins amount bound to the relative increase in diameter of the different particle aggregated sizes in **paper I**, showed that the smallest sized particle Fe_3O_4 bound the highest amount of proteins. Measurements of the protein corona formations revealed that although the hydrodynamic particle mean size was similar for Fe_2O_3 and UF_{WF} particles, Fe_2O_3 bound twice the amount of proteins than the UF_{WF} particles. Thus the chemical composition of the primary particles along with the primary particle sizes of the agglomerates might determine the specific surface area available for binding (surface area per mass unit). These results suggested that parameters such as chemical composition, agglomerated particle sizes along with the particle sizes, could determine the binding capacity of different particles.

Biological findings

Protein identifications in pooled samples analyzed with shotgun proteomics

The total protein concentration in the nasal lavage fluid did not significantly differ between the two groups studied in **paper III** and **paper IV** (**table 2**).

Table 2. Total protein concentration in nasal lavage.
The total protein concentration (μg/mL) in nasal lavage samples collected during filtered air exposure in **paper III** and **IV**.

	NL 1	**NL 2**	**NL 3**
	Total protein concentration (μg/mL); mean; median (min-max)		
Paper III	137;129 (88-191)	177;170 (91-239)	204;193 (101-358)
Paper IV	151;127 (87-361)	201;180 (126-481)	217;180 (124-480)

The same amounts of total protein content were analyzed with the shotgun method of the pooled samples analyzed in **paper III** and **IV**. The discovery based protein identification from pooled samples from welders with lower respiratory symptoms generated 336 proteins in **paper III** while the pooled samples of the healthy volunteers in **paper IV** generated 211 identified proteins. More than 100 proteins were detected in **paper III** compared to the number of proteins identified in **paper IV**. In shotgun proteomics, the complexity and the high dynamic range of a sample will to some extent affect the number of identified proteins. The difference in the number of identified proteins between **paper III** and **paper IV** could due to a number of factors, such as differences in the mucosa protein abundance between the study groups, or the different exposures.

Proteome changes in individual samples analyzed with targeted proteomics

Qualitative determination (relative quantification)

In **paper II** 175 proteins were identified to be significantly altered ($p < 0.05$) after a persulfate challenge of female hairdressers with and without work related rhinitis and a group of atopic females. After adjusting for multiple statistical tests, 54 proteins were still significantly altered ($p < 0.0023$) in at least one of the groups. The largest number of significantly altered proteins was found in the asymptomatic group, 44 proteins, compared to six proteins for the symptomatic group, and 17 proteins for the atopic group. However, several of these proteins showed similar trends ($p < 0.05$) in all groups. Differences between the groups, although not statistically significant, were seen for mucin-5b, interleukin-1 receptor antagonist protein (IL-1RA), desmoplakin, Ig alpha-1 chain c region (IGHA1), glutathione-S-transferase P (GSTP1), and triosephosphate isomerase (TPIS). In **paper III**, 46 proteins were identified to be significantly altered ($p < 0.05$) by welding fume particle exposure when analyzed with LMM, and 32 remained significant ($p < 0.03$) after FDR correction. Fifty-six proteins were identified to be significantly altered ($p < 0.05$) by welding fume particle exposure when analyzed with Wilcoxon signed rank test and 35 remained significant ($p < 0.036$) after FDR correction. Thirty proteins could be identified with both statistical methods. In **paper IV**, data analyses revealed 71 significantly altered ($p < 0.05$) proteins by diesel exhaust exposure with LMM, and 68 proteins remained significant ($p < 0.043$) after FDR correction. Seventy-six proteins and one *in vivo* peptide degradation product (collagen 4 A1) were identified to be significantly altered ($p < 0.05$) with Wilcoxon signed rank test after diesel exhaust exposure, and 73 remained significant ($p < 0.046$) after correction for FDR. Forty-nine proteins could be identified with both statistical methods.

Peptide oxidation and *in vivo* peptide degradation products were identified to be significantly altered in **paper II** and **paper IV**. The oxidation degree increased significantly for albumin peptides containing oxidized (+32 Da) trp214, 5h after the challenge in the asymptomatic group. The same trend was seen in all groups, and no significant differences in oxidation degree were detected between the groups. When data from all groups were included in the same statistical analyses, a significant increase was identified at 2h and 5h after the persulfate challenge in **paper II**. The *in vivo* peptide degradation products from collagen 4 A1 were identified in **paper IV** to decrease after diesel exhaust exposure**.**

Thirty-one proteins were identified in at least two of the three papers to be significantly altered after the respective exposures in **paper II-IV** (**table 3**) identified with LMM statistical test. Among these proteins caspase -14 was the only protein that was identified in all groups and all exposures to be associated with the different exposures.

Quantitative determination (absolute quantification)

In **paper III** and **IV**, MMP9, A1AT and A2M were absolutely quantified. MMP9 was the only protein that was significantly altered by the exposure in both papers. The absolute quantification of the individual samples showed that 90% of all samples had a concentration higher than the LOD for A1AT; 95% of all samples had a concentration higher than the LOD for A2MG, and 88% of all samples had a concentration higher than the LOD for MMP9 in **paper III**. The data obtained from the eleven studied welders suggested that the MMP9 concentration seem to be correlated with the welding years, but just for the ones that have been in the occupation for 4-26 years (**figure 5**). The absolute quantification showed that 99% of all individual samples had a concentration above LOD for A1AT, 99% had a concentration above LOD for A2MG and 98% had a concentration above LOD for MMP9 in **paper IV**. All three proteins did significantly increase after exposure compared to when exposed to filtered air. Comparing the absolute quantity of MMP9, A1AT and A2MG between the healthy volunteers in **paper IV** and the welders with lower respiratory symptoms in **paper III** revealed that the healthy male volunteers in **paper IV** had higher A1AT and A2MG compared to the welders with lower airway symptoms in **paper III (table 4-5)**. No significant difference was detected between the male and female volunteers in **paper IV** regarding MMP9, A1AT and A2MG concentration.

Table 3. Significantly changed proteins in common in paper II-IV with the linear mixed model.

Nasal lavage proteins from the different papers were analyzed with SRM. There were 31 proteins that were identified to be significantly altered in at least two of the three **papers II-IV**. In **paper II**, the proteins that were identified as significantly altered were the ones that had an increased or decreased protein level after the persulfate challenge compared to the sample taken before the challenge. In **paper III** and **IV**, the proteins that were identified as significantly altered were the ones that had differential protein abundance between the samples taken at the exposure day compared to the samples taken at the filtered air exposure day. ns: not significant, (-) were not included in the SRM method of. T: trend of change (0.0023> p <0.05). W: significant (p <0.05) only with Wilcoxon signed rank test. Purple indicates an increase and blue indicates a decrease.

	paper II			**paper III**	**paper IV**
	Hairdressers Symptomatic	Hairdressers Asymptomatic	Atopic Without work related bleaching powder exposure	Welders with lower airway symptoms	Healthy Volunteers
alpha-1-antichymotrypsin	ns		T	ns	
alpha-1-antitrypsin	T		ns	ns	
alpha-2-macroglobulin	ns	ns	ns		
antileukoproteinase	ns		T	ns	
antithrombin-III	ns	ns	ns		
caspase-14					
cofilin-1	T		T	ns	
complement factor b	ns		ns	ns	
desmoplakin	ns		T		ns
dystroglycan	-	-	-		
Ezrin	ns	T		W	
fatty acid-binding protein			T	-	
fibrinogen alpha chain		T		-	
fibronectin	-	-	-		
galectin-3-binding protein	T		T		W
glutathione s-transferase p	ns			ns	
hemopexin	ns		T		
interleukin-1 receptor antagonist	ns		ns		W

lipocalin-15	ns		T		ns
mammaglobin-b	ns	ns	ns		
matrix metalloproteinase-9	-	-	-		
moesin	ns		ns	W	
myeloperoxidase	ns	ns	ns		
neutrophil elastase	ns	T		ns	
polymeric immunoglobulin receptor	ns		T	-	
profilin-1	-	-	-		
prosaposin	-	-	-		
uteroglobin				W	
vimentin	ns		T		
wap four-disulfide core domain protein	T	T	T	W	
zymogen granule protein 16 homology		T	T		ns

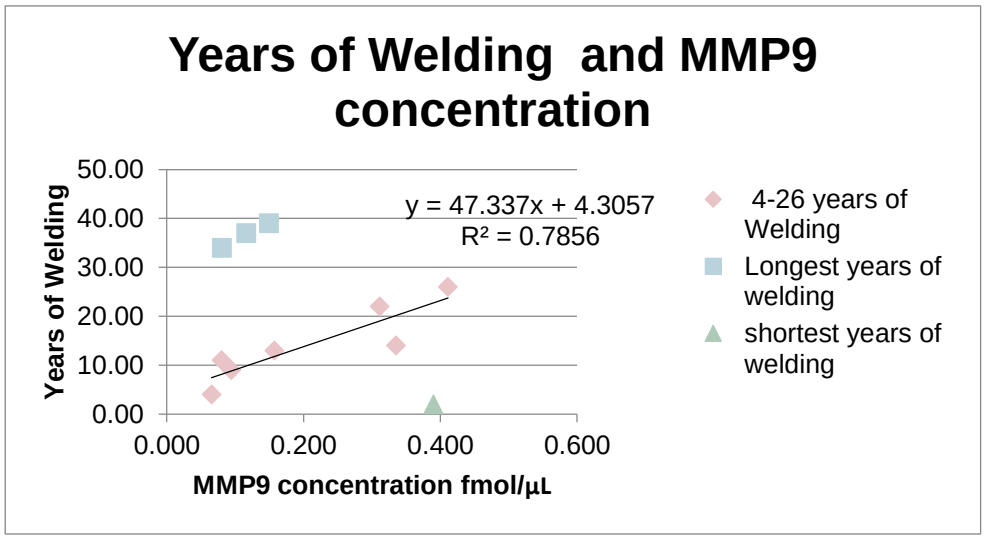

Figure 5.
Correlating years of welding with MMP9 concentration. There seems to be a correlation between the years of welding and the MMP9 concentration in the upper airways for welders with lower respiratory symptoms that have been in the occupation for 4-26 years.

Table 4. Concentration (fmol/µL) (mean: median (min-max)) of A1AT, MMP9 and A2MG in paper III and IV.
A: exposure of welding fume particles in **paper III** and diesel exhaust in **paper IV**. B: filtered air exposure

	Paper		**NL 1**	**NL 2**	**NL 3**	**Gender**			
A1AT	III	A	0.51: 0.37 (0.23-1.28)	0.50: 0.37(0.23-1.6)	0.44: 0.52 (0.14-0.94)				
		B	0.67: 0.51 (0.30-2.12)	0.52: 0.41 (0.21-1.84)	0.56: 0.45 (0.2-1.48)				
	IV	A				female	1.8: 0.78 (0.48-8.7)	1.9: 0.74 (0.59-9.31)	1.5: 0.69 (0.35-7.2)
			1.4: 0.85 (0.38-8.7)	1.4: 0.86 (0.59-9.3)	1.17: 0.7 (0.34-7.2)	male	0.9: 0.87 (0.38-1.45)	0.95: 0.88 (0.62-1.43)	0.87 :0.69 (0.34-2.33)
		B	1.1: 0.1 (0.37-2.4)	1.1: 0.84 (0.28-4.9)	0.84: 0.57 (0.31-3.5)	female	1.02: 0.95 (0.37-2.09)	1.26: 0.68 (0.28-4.92)	0.96: 0.4 (0.33-3.47)
						male	1.16: 1.0 (0.42-2.36)	1.01: 0.9 (0.46-2.04)	0.72: 0.68 (0.31-1.35)
A2MG	III	A	0.52: 0.43 (0.09-0.13)	0.42: 0.20 (0.08 -1.32)	0.35: 0.29 (0.05-0.95)				
		B	0.50: 0.32 (0.16-1.13)	0.47: 0.24 (0.09-1.55)	0.50: 0.51 (0.11-1.44)				
	IV	A				female	1.12: 0.83 (0.31-2.85)	1.38 : 0.83 (0.49-4.36)	1.23: 1.10 (0.34-2.68)
			1.1: 0.9 (0.31-2.9)	1.3:0.87 (0.27-4.4)	1.1: 0.92 (0.34-2.7)	male	0.98: 0.93 (0.34-1.65)	1.11: 0.94 (0.27-3.34)	0.98: 0.83 (0.34-2.31)
		B	1.1: 0.97 (0.02-2.5)	1.1:0.89 (0.37-2.2)	0.97: 0.85 (0.26-2.6)	female	1.01: 0.96 (0.02-2.48)	1.16: 0.88 (0.44-2.17)	1.17: 0.99 (0.26-2.61)
						male	1.13: 1.04 (0.65-2.36)	0.93: 0.96 (0.37-1.75)	0.77: 0.81 (0.41-1.17)
MMP9	III	A	0.26: 0.14 (0.02-0.61)	0.14: 0.11 (0.02-0.37)	0.19: 0.13 (0.02-0.36)				
		B	0.16: 0.15 (0.02-0.42)	0.16: 0.11 (0.03-0.52)	0.18: 0.16 (0.08-0.4)				
	IV	A				female	0.10: 0.04 (0.02-0.40)	0.15: 0.09 (0.04-0.35)	0.14: 0.08 (0.05-0.39)
			0.13: 0.065 (0.02-0.4)	0.17: 0.11 (0.04-0.39)	0.16: 0.11 (0.05-0.46)	male	0.16: 0.15 (0.04-0.40)	0.20: 0.15 (0.07-0.39)	0.17: 0.12 (0.05-0.46)
		B	0.17: 0.13 (0.02-0.46)	0.16: 0.1 (0.04-0.37)	0.13: 0.12 (0.01-0.31)	female	0.13: 0.11 (0.02-0.46)	0.13: 0.08 (0.04-0.35)	0.14: 0.09 (0.01-0.31)
						male	0.17: 0.15 (0.04-0.37)	0.19: 0.11 (0.07-0.37)	0.11: 0.11 (0.02-0.21)

Table 5.

Comparison of A1AT, A2MG and MMP9 concentrations between the male volunteers in **paper IV** and the welders with lower airway symptoms in **paper III** from the blank exposure samples at the different sampling times, statistically evaluated with the Mann-Whitney U test. A significant difference was assumed to be a p- value <0.05. * p <0.05, **p <0.01

	NL 1	**NL 2**	**NL 3**
	p-value		
A1AT	0.031*	0.006**	0.24
A2MG	0.004**	0.016*	0.022*
MMP9	0.710	0.321	0.079

Pathway analyses

In **paper I**, protein network analyzed by IPA of the high-affinity proteins for F_{WF} and Fe_2O_3 showed common connections for nuclear factor kappa beta (NFκB) and tumor necrosis factor alpha (TNF-α), while interleukin-6 (IL-6) showed a connection for UF_{WF}, indicating that such binding can influence an inflammatory process. No such connections were found for Fe_3O_4. In **paper II**, the significantly changed proteins were associated with acute phase response signaling, communication between innate and adaptive immune cells and oxidative stress response. Lists with nine proteins that appeared to be affected by the persulfate challenge and should be followed up have previously been associated with tissue damage, inflammatory disease, oxidative stress, suppression of inflammation and epithelial barrier integrity (**table 6**). In **paper III**, the significantly changed proteins were associated with, inflammatory response, lipid signaling, suppression of inflammation and involvement in extra cellular matrix disruption. Several proteases were associated with the welding fume particle exposure, among these, metalloproteases were indicated to be important proteins involved in the acute phase and lipid signaling. Connective tissue proteins and proteins controlling the degradation of such tissues, including two different matrix metalloprotease proteins, MMP8 and MMP9, were among the significantly changed enzymes and identified as important key players in the pathways. In **paper IV**, the significantly changed proteins were associated with inflammatory response, lipid signaling, oxidative stress, cell migration, cell proliferation and cell-adhesions and the coagulation signaling. The inflammatory response indicated to be induced through Th2 signaling.

Table 6,

Pathway analyses of the significantly changed proteins detected in **paper II-IV**. The significantly changed proteins in the different studies showed associations with different pathways. Pathway analyses identified common signaling mechanisms shared between the proteins. If several proteins were identified in the same pathway, then that pathway was defined by a higher z-score. The pathway analyses also defined the over-represented regulators connected to the significantly changed proteins.

	paper II	**paper III**	**paper IV**
Pathways	Acute phase signaling (0.816) Rhoa signaling Communication between innate and adaptive immune cells	Acute phase response signaling (0.38) LXR/RXR activation (1.26)	Acute phase response signaling (1.73) LXR/RXR activation (1.94) Production of nitric oxide and reactive oxygen species in macrophages (2.24) ILK signaling (1) Rhoa signaling (2) Coagulation signaling (-0.82)
Regulators	For the asymptomatic group: TNF, IL6 (positive) IGG, CST5 (negative)	IL-6,NEUROG1,SYVN1, TNF, estrogen receptor (positive) TGFB1, HIF1A, STAT3, IL-1B (negative)	IL6,IL1A,IL-1B,TNF,TGFB1, CD44, TP53, NFKB (positive) CST5, MAPK, MGEA5 (negative)
mediators associated with different respiratory diseases, e.g. asthma, COPD and fibrosis	For the asymptomatic group: IL1RN, TNF, IL6	MMP9, MMP8, IL6, IL1B, TNF, IFNG, IL1RN	IL-4, IL-6, IL-1A, MMP9, IL-1B, TNF, IL-13
Important proteins	CASP14, DMBT1, DSP, GSTP1, IL1RN, MUC5B, PRDX, SCGB1A1, WFDC2	MMP9, MMP8 Altered level of extracellular matrix proteins	IL-4, IL-13, IL-5,CCL11, MMP9 Might be Th2 induced immune response

Methodological aspects

Preparation of particle suspension.

The particles were suspended in liquid and sonicated to obtain a homogeneous particle suspension in **paper I**. Following suspension, the agglomerated size differed for the different particles. The two welding fume particle fractions F_{WF} (0.1- 2.5 μm) and UF_{WF} (less than 0.1 μm) shifted to an agglomerated mean hydrodynamic particle size of 130 nm for F_{WF} and 99 nm for UF_{WF}. The two iron oxides Fe_2O_3 (20-40 nm) and Fe_3O_4 (8 nm) particles shifted to 100 nm for Fe_2O_3

and 26 nm Fe_3O_4. In a true exposure scenario the sizes of the agglomerates may be quite different and thus the protein corona may also differ.

Normalization

To remove the shift or noise in the data resulting from a systemic or random shift in instrument performance and measurement, different approaches were conducted in **paper II** compared to **paper III** and **IV**. The median of all protein fold changes for a subject and for each time-point was calculated and used as the correction factor in **paper II**. This normalization was based on the assumption that the majority of the proteins do not change in abundance. This can be a rough assumption to encounter when fewer proteins are being targeted. Proteins that were targeted in **paper III** and **IV** were based on a hypothesis that the majority of the proteins would be changed after the different exposures applied in **paper III** and **IV**. Therefore, normalization for a systemic or random shift in instrumentation and performance was conducted by isotopically labeled peptides from MMP9, A1AT and A2MG used separately as global normalizing peptides for the relative quantification. The different normalization strategies could have affected the results detected in the different papers.

Statistical evaluation on the peptide level for individual samples

Statistical analyses were conducted on the median ratio of the peptide representing a protein in **paper II** since the median value is more robust for outliers. Which means the proteins that were represented by an odd number of peptides, the statistical test will only encounter one peptide in the statistical analysis. This resulted in not statistically evaluating all detected peptides. In **paper III** and **IV**, the statistical analyses were therefore conducted on peptide levels for all peptides. Both **paper III** and **IV** revealed that a small number of proteins were represented to be significantly changed by more than one peptide. But the majority of the peptides, representing a protein, showed the same pattern of change. So the results could still be reliable.

Analytical sensitivity

In **paper III,** the LOD was 0.043 fmol/µl for MMP9, 0.205 fmol/µl for A1AT and 0.075 fmol/µl for A2MG. In **paper IV** the LOD was 0.013 fmol/µl for MMP9, 0.06 fmol/µl for A1AT and 0.12 fmol/µl for A2MG. The sensitivity was in the

lower fmol-range and for some protein in the amol-range, which indicates a high sensitivity.

Quality of SRM data

In **paper II**, 78% of the measured peptides had a CV of less than 20%. In **paper III**, 69% of the SRM assays had a CV of less than 20%. When normalizing the total area of each SRM assay to the total area of the isotopically labeled peptides belonging to MMP9, A1AT, A2MG APOB, respectively, 84%, 83%, 72%, 73% and 84% had a CV of less than 20%. In **paper IV**, 55% of all SRM assays had a variation of less than 20% and 76% of all SRM assays had a variation less than 30 %. When normalizing with the isotopically labeled reference peptides MMP9, A1AT and A2MG (927 and 618) 70%/ 72%/72% and 66% of the data had a variation less than 20%, and 78%/80%/84% and 80% had a variation of less than 30%. 80% of all SRM assays were detected in **paper IV**. **Paper III** and **IV** targeted many proteins with low abundance, e.g. proteins from the extra cellular matrix and structural proteins and cytokines.

Key findings

Paper I, protein-corona: measurements showed that the amount of protein bound to particles correlated with the relative size increase of the aggregates, suggesting that the surface area was associated with the binding capacity. Relative quantitative mass spectrometric and gel-based analyses showed differences in the protein content of the coronas. High-affinity proteins were further assessed for functionality analysis, revealing that the type of particle influenced the functionality of the bound protein in different ways.

Paper II, persulfate exposure: analyses of the nasal lavage proteins in a persulfate challenge revealed 54 significantly changed protein levels. Several of the altered proteins were involved in inflammatory responses, oxidative stress, epithelium integrity, and dermatological disorders. Differences in protein changes were detected between the different groups. It is suggested that nine proteins from that protein list (caspase-14, DMBT1, uteroglobin, IL-1RN, desmoplakin, GSTP1, mucin- 5b, peroxiredoxin and WFDC2) should be further investigated after persulfate exposure. Furthermore, an albumin peptide containing oxidized tryptophan increased after the challenge indicated that such peptides may be useful as oxidative stress biomarker.

Paper III, welding fume particle exposure: analyses of the individual samples revealed 30 significantly altered protein levels. These proteins were associated with two main pathways; LXR/RXR activation and acute phase response signaling. Connective tissue proteins and proteins controlling the degradation of such tissues, including two different matrix metalloprotease proteins, MMP8 and MMP9, were among the significantly altered enzymes and identified as important key players in the pathways. These proteins are known to play an important role in degrading ECM proteins and activating other various inflammatory proteins.

Paper IV, diesel exposure: the comprehensive protein level analyses revealed in total 49 significantly altered proteins due to diesel exhaust exposure. These were associated with several pathways e.g. acute phase signaling and LXR/RXR activation, a pathway indirectly related to the activation of lipid metabolism and transport. Furthermore, pathways associated with oxidative stress, cell migration, cell proliferation and cell-adhesions were also induced. Data suggested that the coagulation system was suppressed. The inflammatory response was suggested to be induced through Th2 signaling.

General Discussion

Epidemiological studies have shown an association between exposure to airborne particles and development of different health effects. Mechanisms such as oxidative stress and inflammation have been suggested to play a role in the pathogenesis of the induced effect, and those mechanisms have been investigated in experimental studies using a selection of specific biomarkers [22, 35, 56, 57, 80-83]. Still, there is a need for more comprehensive analyses to explore if other mechanisms are involved. In order to capture individuals who are about to develop a disease associated with particle exposure, it is important to develop biomarkers to be used for diagnostic and prognostic purposes reflecting the different stages of the induced effect. In this thesis, proteomic analyses of nasal lavage samples collected from experimental studies were performed in order to comprehensively study the induced biological responses associated with particle exposure. The results of this thesis help to better understand the response induced in the upper airways by occupational and environmental particle exposures, and to further elucidate the underlying mechanisms.

The analyses of the protein corona formed between nasal lavage proteins and welding fume particles in **paper I**, revealed that the protein corona formation depends upon the particle size, chemical composition, agglomerated size and total surface area represented by the primary particles. Some of these parameters can influence the dose metric suitable for the assessment of biological response. The findings in **paper I** support previous studies [10, 20, 84] that surface area is important to include in assessment of a biological response.

Furthermore the results showed that different proteins differed in binding affinity. Functionality testing was conducted for one antiprotease (antileukoproteinase) that bound to some particles with high affinity. It was shown that the capacity of this protein, which is to inhibit elastase from digesting elastin, was partially inhibited by the particle binding. This factor is important to consider when evaluating the biological response following particle exposure.

Inflammation has been suggested to play a major role in the pathogenesis of particle induced diseases [85-87] and the results in this thesis supported that inflammation was involved in the short term response. The involvement of inflammatory markers were determined in all papers as a number of acute phase proteins, as well as cytokines, were identified in nasal lavage associated with the

different particle exposures. Hemopexin was one of the acute phase proteins that increased in all three **papers II-IV**. Serum amyloid A 1 was increased in **paper III**, in response to welding fume particles, and IL-4 and IL-13, were increased in **paper IV**, in response to diesel exhaust.

The results in this thesis indicated that the regulation or remodeling of the extracellular matrix is affected by persulfate, welding fume particles and diesel exhaust exposures and proteases were revealed to be key regulating mediators in the induced effect. Several proteases were differently changed, such as MMP9, MMP8, Caspase-14 and serine protease HTRA1, in **paper II-IV**. Proteases have been suggested to play an important role in the pathogenesis of different respiratory diseases [88-91].

MMPs have been suggested to play an important role in the pathogenesis of respiratory diseases such as asthma, fibrosis and COPD diseases [89, 92-94]. Increased levels and activity of MMP9 have previously been detected in patients with COPD, emphysema and in smokers. Studies have shown that exposure to persulfates, welding fume or diesel exhaust is associated with an increased risk of development of respiratory diseases e.g. asthma, fibrosis and COPD [25, 27, 53, 83]. MMP9 concentration in the nasal lavage was significantly altered in **paper III** and **IV** and levels of other proteases like MMP8 and serine protease HTRA1 were altered in **paper III**. MMP9 is a protease that cleaves collagen and elastin and other extracellular matrix proteins [95]. The levels of caspase -14 were significantly altered in all groups in **paper II-IV**. This protein is thought to be involved in the degradation of profilaggrin into filaggrin, which is a structural protein, found in the extracellular matrix and is important for hydration of the epidermis and skin barrier function [96]. The function of caspase-14 in the airways is unknown but it has been proposed that this protein plays a role in epithelium integrity [97].

In order to counterbalance the excessive activity of different proteases in the respiratory system, a pool of anti-proteases are present in abundance in the lungs e. g. A1AT, A2MG, alpha -1- antichymotrypsin (SERPINA3), tissue metalloproteinase inhibitors 1 (TIMP) and antileukoproteinase. The ratio between specific enzymes and anti-proteases has proven crucial in the airway remodeling [91, 98-101]. The imbalance between MMP9 and TIMP1 has previously been shown to lead to thickened airways with restricted airflow [98]. Low sputum MMP9 over TIMP1 ratios correlate with decreased FEV_1 for patients with asthma and COPD [93]. Too low or too high protease over anti-protease could be the cause of the development of negative health effects. Several anti-proteases were significantly altered in all **papers II-IV**. A1AT and SERPINA3 increased in the asymptomatic group in **paper II**, a decrease of TIMP1 in **paper III**, an increase of antileukoproteinase in **paper IV** and a decrease in **paper II** and an increase of

A2MG, A1AT and SERPINA3 in **paper IV**. The data evaluation of the absolute concentration revealed that the healthy volunteers in **paper IV** had a significantly higher concentration of A2MG and A1AT compared to the welders with lower respiratory symptoms in **paper III**. This suggests, the concentration of different anti-proteases, may be reduced due to earlier welding fume exposure. It is also possible that the functionality of anti-proteases could be altered by the particle exposure. Additionally, the inhibited functionality of antileukoproteinase detected in **paper I**, could result in an excessive protease activity. MMP9 concentration in nasal lavage showed a positive correlation with the lung function, FEV_1% and FVC%, of the welders with lower respiratory symptoms in **paper III**. This correlation could not be detected in **paper IV** between the MMP9 concentration and FEV_1% and FVC% for the healthy volunteers. The MMP9 levels between the two study groups in **paper III** and **IV** were not significantly different. The correlation detected in **paper III** could therefore be due to an imbalance that might have occurred between the protease and the anti-protease concentration. This is possibly caused by the prolonged welding exposure.

Several extracellular proteins such as desmoplakin, moesin, ezrin and fibronectin, were identified to have a differentially changed protein level after exposure to different particles. These protein complexes play a vital role in maintaining the structural integrity of the epithelium. Proteases are known to cleave and remodel extracellular matrix proteins. They play a central role in normal tissue structure to maintain the balance between formation and degradation of extracellular matrix proteins and are involved in respiratory tract remodeling [98, 102, 103]. If this balance is disrupted, a progressive remodeling could lead to significant functional impairment, resulting in respiratory diseases such as fibrosis, asthma and COPD [89, 91, 101, 104]. The levels of desmoplakin in **paper II** and **III** appeared to be differentially changed. Desmoplakin is a desmosomal protein critical to cell–cell adhesion in a variety of cell types and important in the healing of wounds and epithelial barrier function [105]. Increased expression of desmoplakin has been suggested to have a potential role in the pathogenesis of idiopathic pulmonary fibrosis [106]. The increased or decreased levels of structural proteins in nasal lavage fluid might be the result of tissue damage induced by the different exposures, or a response from the activated proteases cleaving the tight junctions of the epithelial cells so that permeability increases. A significant correlation between the concentration of MMP9 in nasal lavage and lung function (FEV_1 and FVC %) was detected in **paper III**. This could be due to the start of remodeling impairment by the prolonged welding fume exposure.

The protease activity is normally induced by the inflammation [107, 108] and inhibited indirectly by an immunosuppressive response [109]. Several immunosuppressive proteins were also detected in all three **papers II-IV**, e.g. IL-1RA and uteroglobin. Uteroglobin decreased in all groups in **paper II**, while IL-

1AR increased in the asymptomatic group. Both uteroglobin and IL-1AR were increased in **paper III** for the welders with lower airway symptoms and decreased in **paper IV** for the healthy volunteers. Immunosuppressive proteins have been suggested to be involved in several respiratory illnesses [110, 111]. Elevated levels of IL-1RA have been found in patients with idiopathic pulmonary fibrosis and asthma [112] and a low level of IL-1RA has been associated with a more severe inflammation [113, 114]. Both IL-1RA and uteroglobin have been suggested as anti-therapeutic mediators in allergic rhinitis [115-118].

This thesis suggests that proteases, anti-proteases, disruption of the extracellular matrix, inflammation and immunosuppression all contribute to the induced effects by occupational and environmental particles. A prolonged exposure could possibly induce an imbalance between the regulatory mechanisms causing the symptom.

The study designs of the different papers in this thesis differed in several ways that could affect the detected response, e.g. healthy and symptomatic subjects, exposure, dose, duration of exposure and sampling times. This made it hard to directly compare the specific results obtained from the different exposures. Still, general conclusions could be drawn regarding particle exposure and the induced effects, such as changed levels of proteases, anti-protease, extracellular matrix proteins, immune response proteins and immunosuppressive proteins were associated with different particle exposures. Different protein changes were detected associated with different particle exposures. The induced effect differed between different groups exposed to the same particles.

Still, there is a need for well-defined exposures, regarding e. g. particle size, chemical composition, aggregated form and surface area of the particle, as well as well-defined groups, regarding current health, symptoms and gender to be able to compare the exposures with each other. In epidemiological studies where co-exposures exist for, e.g welders, it is more difficult to evaluate if the detected response is induced from, e.g. gases or the welding fume particles. An advantage in controlled experimental studies such in the study in **paper III** is that it could be concluded that the particle fraction alone could induce an inflammatory response.

There is a large inter individual variation in protein levels in the nasal lavage fluids [119]. Therefore a major strength of the study design in **paper II-IV** was that each person acted as their own control, by comparing the samples for each individual from before and after exposure. Additionally, a blank exposure with filtered air was added in **paper III** and **IV** to rule out any induced protein alterations that could be induced by the nasal lavage sampling or diurnal variation.

Absolute quantification was also measured for some proteins, MMP9, A1AT and A2MG, with the advantage of being able to compare between the healthy male volunteers in **paper IV** and the welders with lower airway symptoms in **paper III**.

The changed protein levels after an exposure might be driven by a cascade of changed proteins, with different protein changes appearing at different time points [120, 121]. In this thesis, the sampling is taken just a few times after the exposure. This will make the appearance of all the altered proteins impossible to find from the few sampling points.

The SRM approach facilitated high throughput screening of a large number of nasal lavage proteins with both a relative quantification and absolute quantification with good reproducibility in all papers. A proteomic screening strategy was applied in **paper I and II** by targeting as many proteins as possible. The targeted method contained SRM assays for approximately 247 proteins. They were divided into four methods, and each was run separately. But dealing with this many proteins resulted in a time and labor consuming process when evaluating the results. This approach was successful when fewer samples were analyzed, but when more samples were analyzed larger variations in retention time appeared. Additionally, even though a large number of proteins (247) were targeted, the method was not complete. The present knowledge of the identified proteins in the nasal lavage cavity exceeds the present number detected in **paper II** [66, 122-125]. Over 900 [125] proteins have been identified in a recent study, thus many proteins in nasal lavage fluid are not included in the method applied in **paper I** and **II**. The method probably targets the most abundant proteins in nasal lavage fluid. Also, since the method was constructed using samples from healthy donors [76], some disease or exposure related proteins may be missed. However, such proteins can be identified with untargeted analyses of pooled samples at the beginning of the study.

Although the shotgun approach is conceptually simple, it results in greatly increased complexity of the generated peptide mixture, requiring highly sensitive and efficient separation. Furthermore, the limited dynamic range of mass spectrometric analysis only allows for the peptides present at high relative abundance to be preferentially sampled, while information regarding the proteins represented as low abundance peptides in the complex mixture is commonly not obtained. Therefore, the proteins that were identified to be changed with the shotgun method were further investigated with pathway analyses to generate a hypothesis of the induced effects. Proteins that were associated with these pathways but not detected with the discovery method were added to the targeted method to comprehensively analyze these pathways in **paper III** and **IV**. Drawbacks with such strategy could be that important information from the proteins not targeted could be lost and that there is a chance of determining the course of the results. However, the protein findings were easier to interpret than in **paper II** and stronger associations with specific pathways were obtained.

In **paper III** and **IV**, the SRM method contained SRM assays for both relative and absolute quantification in the same run. A drawback in the relative quantification in **paper III** and **IV** was the limited statistically agreement of the peptide analyses. There were few proteins that were represented by more than one significantly altered peptide. However, when the changes in the peptides were in line with each other the results were still reliable.

The results revealed that combining untargeted and targeted protein analyses of human nasal lavage fluid proved to be a powerful tool for investigating early responses induced by occupational and environmental exposures.

In **paper I**, the combination of a targeted SRM LC-MS/MS and 2-DE MALDI-TOF-MS showed to be a good combination to quantify the proteins interacting with welding fume particles of different particle sizes. The 2-DE MALDI TOF-MS generated a visual representation of the results which were easy to verify and in good agreement with the targeted method. Previous studies of protein particle interaction focused on the qualitative protein measure [126]. The study in **paper I** allowed a more quantitative protein measure of the proteins interacting with the different particles, by measuring the proteins with a targeted mass spectrometry method developed for nasal lavage proteins. The biological matrix contains complex mixtures of molecules with a large dynamic range [127]. The targeted approach provided a better opportunity for the lower abundant proteins to be quantified revealing their importance in such an interaction.

Pathway analysis has proven to be helpful in interpreting the biological relevance associated with the differentially altered proteins in **paper I-IV**. But a major weakness with pathway analysis is that it is based on prior biological knowledge [79], and if the quality of the identified pathways is low or incomplete, it will have a major impact on the results. But a lot of the information in these databases is constantly updated and refined. Additionally, the quality of the pathway analysis was dependent on the number of proteins that are differentially altered. Few proteins were associated with the different exposures in **paper I** and **II.** This resulted in dispersed and weak associations with different pathways. Stronger associations between the differentially altered proteins and the pathways were recorded in **paper III** and **IV**. This could due to the number of altered proteins in the last two papers, and that a more comprehensive hypothesis based approach was addressed. This indicated that the pathway analyses could be helpful in interpreting the biological relevance of the differentially altered proteins associated with the exposure as long as the sizes of the proteins sets are not small.

Conclusions

From this thesis the following conclusions can be drawn:

- The chemical composition, physical shape, and agglomeration state of the particle influenced the protein–particle interactions. The amount of protein bound to each particle type correlates with the relative size increase of the aggregates, suggesting that the surface area per mass unit determines the binding capacity and that this dose metric is important to include in the assessment of a biological response.
- The functionality of antileukoproteinase was inhibited by the protein – particle interaction, suggesting that the protein binding with high affinity to particles could lose their functionality during binding depending on the particle size. This loss could have an excessive downstream effect depending on the inhibited functionality of the bound protein. This factor is important to consider when evaluating the biological response following particle exposure.
- Combining shotgun and targeted protein analysis revealed to be a powerful tool investigating the early proteome response in the upper airways by occupational and environmental particle exposures.
- Several proteins with biologically relevant functions were significantly altered after persulfate, welding fume and diesel exposure in the different exposure groups. Different types of particle exposures generated different protein changes. The same exposure induced different protein changes in different subgroups. Addressing the importance of well-defined study groups and the importance of including different subgroups to be able to comprehensively evaluate the induced effects. In general, the findings suggested that exposure to persulfate, welding fume particles and diesel exhaust induce an inflammatory response that possibly resulted in tissue damage and tissue remodeling.
- Common proteins detected in all three exposures were proteases, anti-proteases, extracellular matrix proteins, inflammatory and immunosuppressive proteins which all were a part of the effects induced by occupational and environmental particle exposures. Proteases and anti-

proteases were suggested to be key proteins that might be important in tissue damage and the remodeling induced by different particle exposures. This thesis suggested that exposure to particles could possibly be the cause of an induced imbalance between these regulatory mechanisms.

Future perspectives

To further explore the proteome response induced by different particle exposures by repeating the exposure experiments and then monitor the exposures for a longer period of time.

Expand the SRM analysis so it includes all identified proteins, in order to facilitate comparison of different proteins are associated with different particle exposures.

The exposures should be further studied at different concentrations. Correlating different particle parameters e.g. agglomerated state, surface area and chemical composition to the induced effects.

Peptidomics could be carried out in a larger degree correlating the protease, anti-protease activity with the peptide degradation products.

In those exposure experiment absolute quantification of more proteins should be included. If comparison between groups and exposures are needed.

References

1. WHO, *Hazard Prevention and Control in the Work Environment: Airborne Dust*. 1999.
2. WHO, *WHO Guidelines for Indoor Air Quality: Selected Pollutants. Copenhagen: WHO Regional office for Europe*. 2010.
3. Agency, U.S.E.P., *Health assessment document for diesel engine exhaust. Report EPA/600/8-90/057F*. Washington, DC: U.S. Environmental Protection Agency, 2002.
4. EPA, U.S., *Air Quality Criteria for Particulate Matter* U.S. Environmental Protection Agency, Washington, DC, EPA 600/P-99/002aF-bF, 2004, 2004.
5. Davidson, C.I., R.F. Phalen, and P.A. Solomon, *Airborne particulate matter and human health: A review*. Aerosol Science and Technology, 2005. **39**(8): p. 737-749.
6. Kim, K.H., E. Kabir, and S. Kabir, *A review on the human health impact of airborne particulate matter*. Environ Int, 2015. **74**: p. 136-43.
7. Iavicoli, I., V. Leso, L. Fontana, and A. Bergamaschi, *Occupational Exposure to Urban Airborne Particulate Matter: A Review on Environmental Monitoring and Health Effects*. Urban Airborne Particulate Matter, 2011: p. 501.
8. Phillip, C., *Hazardous Chemicals Handbook (2nd Edition)*. 2002: Mexico DF, Mexico : Elsevier.
9. Viana, M., *Indoor and Outdoor Nanoparticles. Determinants of Release and Exposure Scenarios*. The Handbook of Environmental Chemistry: 48. 2016: Springer International Publishing
10. Oberdörster, G., A. Maynard, K. Donaldson, V. Castranova, J. Fitzpatrick, K. Ausman, et al., *Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy*. Particle and Fibre Toxicology, 2005. **2**: p. 8-8.
11. Pak, V.M., M. Powers, and J. Liu, *Occupational Chemical Exposures Among Cosmetologists: Risk of Reproductive Disorders*. Workplace health & safety, 2013. **61**(12): p. 522-529.
12. Ronda, E., B.E. Hollund, and B.E. Moen, *Airborne exposure to chemical substances in hairdresser salons*. Environmental monitoring and assessment, 2009. **153**(1-4): p. 83-93.
13. Albin, M., L. Rylander, Z. Mikoczy, L. Lillienberg, A. Dahlman Hoglund, J. Brisman, et al., *Incidence of asthma in female Swedish hairdressers*. Occupational and environmental medicine, 2002. **59**(2): p. 119-23.
14. Brisman, J., M. Albin, L. Rylander, Z. Mikoczy, L. Lillienberg, A.D. Höglund, et al., *The incidence of respiratory symptoms in female Swedish hairdressers*. American Journal of Industrial Medicine, 2003. **44**(6): p. 673.

15. *Threshold Limit Values for chemical substances and physical Agents and Biological Exposure Indices*. 2008, ACGIH
16. Nilsson, P.T., S. Marini, A. Wierzbicka, M. Kåredal, E. Blomgren, J. Nielsen, et al., *Characterization of Hairdresser Exposure to Airborne Particles during Hair Bleaching*. The Annals of Occupational Hygiene, 2016. **60**(1): p. 90-100.
17. Hedmer, M., J.E. Karlsson, U. Andersson, H. Jacobsson, J. Nielsen, and H. Tinnerberg, *Exposure to respirable dust and manganese and prevalence of airways symptoms, among Swedish mild steel welders in the manufacturing industry*. Int Arch Occup Environ Health, 2014. **87**(6): p. 623-34.
18. Isaxon, C., J. Pagels, A. Gudmundsson, C. Asbach, A. John, T. Kuhlbusch, et al. *Characteristics of welding fume aerosol investigated in three Swedish workshops*. in Journal of physics: Conference series. 2009. IOP Publishing.
19. Isaxon, C., K. Dierschke, J. Pagels, J. Löndahl, A. Gudmundsson, I. Hagerman, et al., *A novel system for source characterization and controlled human exposure to nanoparticle aggregates generated during gas–metal arc welding*. Aerosol Science and Technology, 2013. **47**(1): p. 52-59.
20. Oberdörster, G., V. Stone, and K. Donaldson, *Toxicology of nanoparticles: A historical perspective*. Nanotoxicology, 2007. **1**(1): p. 2-25.
21. Sager, T.M. and V. Castranova, *Surface area of particle administered versus mass in determining the pulmonary toxicity of ultrafine and fine carbon black: comparison to ultrafine titanium dioxide*. Particle and Fibre Toxicology, 2009. **6**: p. 15-15.
22. Badding, M.A., N.R. Fix, J.M. Antonini, and S.S. Leonard, *A comparison of cytotoxicity and oxidative stress from welding fumes generated with a new nickel-, copper-based consumable versus mild and stainless steel-based welding in RAW 264.7 mouse macrophages*. PLoS One, 2014. **9**(6): p. e101310.
23. Leonard, S.S., B.T. Chen, S.G. Stone, D. Schwegler-Berry, A.J. Kenyon, D. Frazer, et al., *Comparison of stainless and mild steel welding fumes in generation of reactive oxygen species*. Part Fibre Toxicol, 2010. **7**: p. 32.
24. Antonini, J.M., M.D. Taylor, A.T. Zimmer, and J.R. Roberts, *Pulmonary responses to welding fumes: role of metal constituents*. Journal of toxicology and environmental health. Part A, 2004. **67**(3): p. 233-49.
25. Antonini, J.M., *Health effects of welding*. Crit Rev Toxicol, 2003. **33**(1): p. 61-103.
26. OSHA, *Diesel Exhaust/Diesel Particulate Matter*. https://www.osha.gov/dts/hazardalerts/diesel_exhaust_hazard_alert.html, 2017-08-16.
27. Santonen, P.T.a.T., *Diesel Engine Exhaust*. The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and the Dutch Expert Committee on Occupational Safety, 2016. **49(6)**.
28. Moscato, G., P. Pignatti, M.R. Yacoub, C. Romano, S. Spezia, and L. Perfetti, *Occupational asthma and occupational rhinitis in hairdressers*. Chest, 2005. **128**(5): p. 3590-8.
29. Lind, M.-L., M. Albin, J. Brisman, K. Kronholm Diab, L. Lillienberg, Z. Mikoczy, et al., *Incidence of hand eczema in female Swedish hairdressers*. Occupational and environmental medicine, 2007. **64**(3): p. 191-195.

30. Uter, W., A. Pfahlberg, O. Gefeller, and H.J. Schwanitz, *Risk of hand dermatitis among hairdressers versus office workers*. Scandinavian journal of work, environment & health, 1999. **25**(5): p. 450-456.
31. Hammond, S.K., E. Gold, R. Baker, P. Quinlan, W. Smith, R. Pandya, et al., *Respiratory health effects related to occupational spray painting and welding*. J Occup Environ Med, 2005. **47**(7): p. 728-39.
32. Hannu, T., R. Piipari, M. Tuppurainen, H. Nordman, and T. Tuomi, *Occupational asthma caused by stainless steel welding fumes: a clinical study*. The European respiratory journal, 2007. **29**(1): p. 85-90.
33. Antonini, J.M. and J.R. Roberts, *Chromium in stainless steel welding fume suppresses lung defense responses against bacterial infection in rats*. J Immunotoxicol, 2007. **4**(2): p. 117-27.
34. Antonini, J.M., S. Stone, J.R. Roberts, B. Chen, D. Schwegler-Berry, A.A. Afshari, et al., *Effect of short-term stainless steel welding fume inhalation exposure on lung inflammation, injury, and defense responses in rats*. Toxicology and applied pharmacology, 2007. **223**(3): p. 234-45.
35. Erdely, A., J.M. Antonini, S.-H. Young, M.L. Kashon, J.K. Gu, T. Hulderman, et al., *Oxidative stress and reduced responsiveness of challenged circulating leukocytes following pulmonary instillation of metal-rich particulate matter in rats*. Particle and Fibre Toxicology, 2014. **11**: p. 34-34.
36. Antonini, J.M., M.D. Taylor, L. Millecchia, A.R. Bebout, and J.R. Roberts, *Suppression in lung defense responses after bacterial infection in rats pretreated with different welding fumes*. Toxicology and applied pharmacology, 2004. **200**(3): p. 206-18.
37. Sriram, K., G.X. Lin, A.M. Jefferson, J.R. Roberts, R.S. Chapman, B.T. Chen, et al., *Dopaminergic neurotoxicity following pulmonary exposure to manganese-containing welding fumes*. Arch Toxicol, 2010. **84**(7): p. 521-40.
38. Antonini, J.M., A.B. Santamaria, N.T. Jenkins, E. Albini, and R. Lucchini, *Fate of manganese associated with the inhalation of welding fumes: potential neurological effects*. Neurotoxicology, 2006. **27**(3): p. 304-10.
39. Contreras, G.R. and M. Chan-Yeung, *Bronchial reactions to exposure to welding fumes*. Occupational and environmental medicine, 1997. **54**(11): p. 836-839.
40. d'Errico, A., J. Zajacova, A. Cacciatore, A. Baratti, R. Zanelli, S. Alfonzo, et al., *Occupational risk factors for sinonasal inverted papilloma: a case–control study*. Occupational and environmental medicine, 2013. **70**(10): p. 703-708.
41. Castano, R. and E. Suarthana, *Occupational rhinitis due to steel welding fumes*. American Journal Of Industrial Medicine, 2014. **57**(12): p. 1299-1302.
42. Acosta, D., *Cardiovascular toxicology* 2008: Informa Healthcare.
43. Pandya, R.J., G. Solomon, A. Kinner, and J.R. Balmes, *Diesel exhaust and asthma: hypotheses and molecular mechanisms of action*. Environmental Health Perspectives, 2002. **110**(Suppl 1): p. 103-112.
44. Hart, J.E., F. Laden, E.A. Eisen, T.J. Smith, and E. Garshick, *Chronic obstructive pulmonary disease mortality in railroad workers*. Occupational and environmental medicine, 2009. **66**(4): p. 221-6.
45. Hart, J.E., E.A. Eisen, and F. Laden, *Occupational diesel exhaust exposure as a risk factor for COPD*. Current opinion in pulmonary medicine, 2012. **18**(2): p. 151-154.

46. Groneberg-Kloft, B., T. Kraus, A.v. Mark, U. Wagner, and A. Fischer, *Analysing the causes of chronic cough: relation to diesel exhaust, ozone, nitrogen oxides, sulphur oxides and other environmental factors*. Journal of Occupational Medicine and Toxicology 2006. **1**: p. 6-6.
47. Bernstein, D.I., *Diesel exhaust exposure, wheezing and sneezing*. Allergy, asthma & immunology research, 2012. **4**(4): p. 178-83.
48. Rudell, B., T. Sandström, U. Hammarström, M.-L. Ledin, P. Hörstedt, and N. Stjernberg, *Evaluation of an exposure setup for studying effects of diesel exhaust in humans*. International Archives of Occupational and Environmental Health, 1994. **66**(2): p. 77-83.
49. Carlsten, C., A.P. Oron, H. Curtiss, S. Jarvis, W. Daniell, and J.D. Kaufman, *Symptoms in Response to Controlled Diesel Exhaust More Closely Reflect Exposure Perception Than True Exposure*. PLoS ONE, 2013. **8**(12): p. e83573.
50. Diaz-Sanchez, D., M. Penichet-Garcia, and A. Saxon, *Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity*. Journal of Allergy and Clinical Immunology, 2000. **106**(6): p. 1140-1146.
51. Humans, I.W.G.o.t.E.o.C.R.t., *Diesel and gasoline engine exhausts and some nitroarenes*. IARC monographs on the evaluation of carcinogenic risks to humans, 2014. **105**: p. 9-699.
52. Aalto-Korte, K. and S. Mäkinen-Kiljunen, *Specific immunoglobulin E in patients with immediate persulfate hypersensitivity*. Contact Dermatitis 2003. **49**(1): p. 22-25.
53. Mun˜oz, X., M.-J. Cruz, R. Orriols, C. Bravo, M. Espuga, and F. Morell, *Occupational Asthma Due to Persulfate Salts*. Chest. **123**(6): p. 2124-2129.
54. Diab, K.K., L. Truedsson, M. Albin, and J. Nielsen, *Persulphate challenge in female hairdressers with nasal hyperreactivity suggests immune cell, but no IgE reaction*. Int Arch Occup Environ Health, 2009. **82**(6): p. 771-7.
55. Jonsson, L.S., K. Broberg, K. Paulsson, K. Kronholm Diab, and J. Nielsen, *Gene expression in nasal lavage from hairdressers exposed to persulphate*. Int Arch Occup Environ Health, 2009. **82**(10): p. 1261-6.
56. Dierschke, K., C. Isaxon, U.B.K. Andersson, E. Assarsson, A. Axmon, L. Stockfelt, et al., *Acute respiratory effects and biomarkers of inflammation due to welding-derived nanoparticle aggregates*. International Archives of Occupational and Environmental Health, 2017: p. 1-13.
57. Xu, Y., L. Barregard, J. Nielsen, A. Gudmundsson, A. Wierzbicka, A. Axmon, et al., *Effects of diesel exposure on lung function and inflammation biomarkers from airway and peripheral blood of healthy volunteers in a chamber study*. Particle and fibre toxicology, 2013. **10**(1): p. 60.
58. Jarvela, M., P. Kauppi, T. Tuomi, R. Luukkonen, H. Lindholm, R. Nieminen, et al., *Inflammatory response to acute exposure to welding fumes during the working day*. International journal of occupational medicine and environmental health, 2013. **26**(2): p. 220-9.
59. Mayeux, R., *Biomarkers: Potential Uses and Limitations*. NeuroRx, 2004. **1**(2): p. 182-188.

60. Silins, I. and J. Högberg, *Combined toxic exposures and human health: biomarkers of exposure and effect*. International journal of environmental research and public health, 2011. **8**(3): p. 629-647.
61. Council, N.R., in Biologic Markers in Pulmonary Toxicology. 1989, National Academies Press
62. Cole, A.M., P. Dewan, and T. Ganz, *Innate Antimicrobial Activity of Nasal Secretions*. Infection and Immunity, 1999. **67**(7): p. 3267-3275.
63. Pitrez, P.M., S. Brennan, S. Turner, and P.D. Sly, *Nasal wash as an alternative to bronchoalveolar lavage in detecting early pulmonary inflammation in children with cystic fibrosis*. Respirology (Carlton, Vic.), 2005. **10**(2): p. 177-82.
64. Bartlett, J.A., M.E. Albertolle, C. Wohlford-Lenane, A.A. Pezzulo, J. Zabner, R.K. Niles, et al., *Protein composition of bronchoalveolar lavage fluid and airway surface liquid from newborn pigs*. American Journal of Physiology - Lung Cellular and Molecular Physiology, 2013. **305**(3): p. L256-L266.
65. Kosanam, H., M. Sato, I. Batruch, C. Smith, S. Keshavjee, M. Liu, et al., *Differential proteomic analysis of bronchoalveolar lavage fluid from lung transplant patients with and without chronic graft dysfunction*. Clinical biochemistry, 2012. **45**(3): p. 223-30.
66. Benson, L.M., C.J. Mason, O. Friedman, H. Kita, H.R. Bergen, and D.A. Plager, *Extensive Fractionation and Identification of Proteins within Nasal Lavage Fluids from Allergic Rhinitis and Asthmatic Chronic Rhinosinusitis Patients*. Journal of separation science, 2009. **32**(1): p. 44-56.
67. Visith, T., *Proteomics of Human Body Fluids: Principles, Methods, and Applications*. 2007: Netherlands : Humana Press Incorporated.
68. Domon, B. and R. Aebersold, *Options and considerations when selecting a quantitative proteomics strategy*. Nat Biotech, 2010. **28**(7): p. 710-721.
69. Lange, V., P. Picotti, B. Domon, and R. Aebersold, *Selected reaction monitoring for quantitative proteomics: a tutorial*. Mol Syst Biol, 2008. **4**: p. 222.
70. Kiyonami, R. and B. Domon, *Selected reaction monitoring applied to quantitative proteomics*. Methods Mol Biol, 2010. **658**: p. 155-66.
71. García-Campos, M.A., J. Espinal-Enríquez, and E. Hernández-Lemus, *Pathway Analysis: State of the Art*. Frontiers in Physiology, 2015. **6**: p. 383.
72. Jin, L., X.-Y. Zuo, W.-Y. Su, X.-L. Zhao, M.-Q. Yuan, L.-Z. Han, et al., *Pathway-based Analysis Tools for Complex Diseases: A Review*. Genomics, Proteomics & Bioinformatics, 2014. **12**(5): p. 210-220.
73. Orton, D. and A. Doucette, *Proteomic Workflows for Biomarker Identification Using Mass Spectrometry — Technical and Statistical Considerations during Initial Discovery*. Proteomes, 2013. **1**(2): p. 109.
74. Rifai, N., M.A. Gillette, and S.A. Carr, *Protein biomarker discovery and validation: the long and uncertain path to clinical utility*. Nature biotechnology, 2006. **24**(8): p. 971-83.
75. Larance, M. and A.I. Lamond, *Multidimensional proteomics for cell biology*. Nature Reviews Molecular Cell Biology, 2015. **16**(5): p. 269-280.
76. Mortstedt, H., M.H. Karedal, B.A. Jonsson, and C.H. Lindh, *Screening method using selected reaction monitoring for targeted proteomics studies of nasal lavage fluid*. J Proteome Res, 2013. **12**(1): p. 234-47.

77. Mitrea, C., Z. Taghavi, B. Bokanizad, S. Hanoudi, R. Tagett, M. Donato, et al., *Methods and approaches in the topology-based analysis of biological pathways*. Frontiers in Physiology, 2013. **4**: p. 278.
78. Kelder, T., B.R. Conklin, C.T. Evelo, and A.R. Pico, *Finding the right questions: exploratory pathway analysis to enhance biological discovery in large datasets*. PLoS biology, 2010. **8**(8).
79. Khatri, P., M. Sirota, and A.J. Butte, *Ten years of pathway analysis: current approaches and outstanding challenges*. PLoS computational biology, 2012. **8**(2): p. e1002375.
80. Alexopoulos, E.C., X. Cominos, I.P. Trougakos, M. Lourda, E.S. Gonos, and V. Makropoulos, *Biological monitoring of hexavalent chromium and serum levels of the senescence biomarker apolipoprotein J/Clusterin in welders*. Bioinorganic chemistry and applications, 2008: p. 420578.
81. Li, H., M. Hedmer, T. Wojdacz, M.B. Hossain, C.H. Lindh, H. Tinnerberg, et al., *Oxidative stress, telomere shortening, and DNA methylation in relation to low-to-moderate occupational exposure to welding fumes*. Environmental and molecular mutagenesis, 2015. **56**(8): p. 684-93.
82. Raulf, M., T. Weiss, A. Lotz, M. Lehnert, F. Hoffmeyer, V. Liebers, et al., *Analysis of inflammatory markers and metals in nasal lavage fluid of welders*. Journal of toxicology and environmental health. Part A, 2016. **79**(22-23): p. 1144-1157.
83. Cosgrove, M. and W. Zschiesche, *Arc welding of steels and pulmonary fibrosis*. Welding in the World, 2016. **60**(2): p. 191-199.
84. Schmid, O. and T. Stoeger, *Surface area is the biologically most effective dose metric for acute nanoparticle toxicity in the lung*. Journal of Aerosol Science, 2016. **99**: p. 133-143.
85. Byrne, J.D. and J.A. Baugh, *The significance of nanoparticles in particle-induced pulmonary fibrosis*. McGill Journal of Medicine : MJM, 2008. **11**(1): p. 43-50.
86. Tetley, T.D., *Inflammatory cells and chronic obstructive pulmonary disease*. Current drug targets. Inflammation and allergy, 2005. **4**(6): p. 607-18.
87. Frampton, M.W., *Inflammation and airborne particles*. Clinics in occupational and environmental medicine, 2006. **5**(4): p. 797-815.
88. Twigg, M.S., S. Brockbank, P. Lowry, S.P. FitzGerald, C. Taggart, S. Weldon, et al., *The Role of Serine Proteases and Antiproteases in the Cystic Fibrosis Lung*. Mediators of Inflammation, 2015. **2015**: p. 10.
89. Kukkonen, M.K., E. Tiili, T. Vehmas, P. Oksa, P. Piirila, and A. Hirvonen, *Association of genes of protease-antiprotease balance pathway to lung function and emphysema subtypes*. BMC Pulm Med, 2013. **13**: p. 36.
90. Gadek, J.E., G.A. Fells, R.L. Zimmerman, and R.G. Crystal, *Role of Connective Tissue Proteases in the Pathogenesis of Chronic Inflammatory Lung Disease*. Environmental Health Perspectives, 1984. **55**: p. 297-306.
91. Greene, C.M. and N.G. McElvaney, *Proteases and antiproteases in chronic neutrophilic lung disease – relevance to drug discovery*. British Journal of Pharmacology, 2009. **158**(4): p. 1048-1058.
92. Grzela, K., M. Litwiniuk, W. Zagorska, and T. Grzela, *Airway Remodeling in Chronic Obstructive Pulmonary Disease and Asthma: the Role of Matrix Metalloproteinase-9*. Arch Immunol Ther Exp 2016. **64**(1): p. 47-55.

93. Hoshino, M., Y. Nakamura, J. Sim, J. Shimojo, and S. Isogai, *Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation*. J Allergy Clin Immunol, 1998. **102**(5): p. 783-8.
94. Hentschel, J., M. Jäger, N. Beiersdorf, N. Fischer, F. Doht, R.K. Michl, et al., *Dynamics of soluble and cellular inflammatory markers in nasal lavage obtained from Cystic Fibrosis patients during intravenous antibiotic treatment*. BMC Pulmonary Medicine, 2014. **14**: p. 82-82.
95. Mohan, R., S.K. Chintala, J.C. Jung, W.V. Villar, F. McCabe, L.A. Russo, et al., *Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration*. J Biol Chem, 2002. **277**(3): p. 2065-72.
96. Rendl, M., J. Ban, P. Mrass, C. Mayer, B. Lengauer, L. Eckhart, et al., *Caspase-14 expression by epidermal keratinocytes is regulated by retinoids in a differentiation-associated manner*. The Journal of investigative dermatology, 2002. **119**(5): p. 1150-5.
97. Denecker, G., P. Ovaere, P. Vandenabeele, and W. Declercq, *Caspase-14 reveals its secrets*. The Journal of Cell Biology, 2008. **180**(3): p. 451-458.
98. Atkinson, J.J. and R.M. Senior, *Matrix metalloproteinase-9 in lung remodeling*. Am J Respir Cell Mol Biol, 2003. **28**(1): p. 12-24.
99. Page-McCaw, A., A.J. Ewald, and Z. Werb, *Matrix metalloproteinases and the regulation of tissue remodelling*. Nature reviews. Molecular cell biology, 2007. **8**(3): p. 221-233.
100. Brew, K. and H. Nagase, *The tissue inhibitors of metalloproteinases (TIMPs): An ancient family with structural and functional diversity*. Biochimica et biophysica acta, 2010. **1803**(1): p. 55-71.
101. Mautino, G., F. Capony, J. Bousquet, and A.M. Vignola, *Balance in asthma between matrix metalloproteinases and their inhibitors*. Journal of Allergy and Clinical Immunology, 1999. **104**(3): p. 530-533.
102. Lu, P., K. Takai, V.M. Weaver, and Z. Werb, *Extracellular Matrix Degradation and Remodeling in Development and Disease*. Cold Spring Harbor perspectives in biology, 2011. **3**(12): p. a005058.
103. Menou, A., J. Duitman, P. Flajolet, J.M. Sallenave, A.A. Mailleux, and B. Crestani, *Human airway trypsin-like protease, a serine protease involved in respiratory diseases*. American journal of physiology. Lung cellular and molecular physiology, 2017. **312**(5): p. L657-l668.
104. Lomas, D.A., *Does Protease–Antiprotease Imbalance Explain Chronic Obstructive Pulmonary Disease?* Annals of the American Thoracic Society, 2016. **13**(Supplement 2): p. S130-S137.
105. Huber, O., *Structure and function of desmosomal proteins and their role in development and disease*. Cellular and molecular life sciences : CMLS, 2003. **60**(9): p. 1872-90.
106. Mathai, S.K., B.S. Pedersen, K. Smith, P. Russell, M.I. Schwarz, K.K. Brown, et al., *Desmoplakin Variants Are Associated with Idiopathic Pulmonary Fibrosis*. American journal of respiratory and critical care medicine, 2016. **193**(10): p. 1151-1160.
107. Dai, H. and R.J. Korthuis, *Mast Cell Proteases and Inflammation*. Drug discovery today. Disease models, 2011. **8**(1): p. 47-55.

108. Qureshi, N., D.C. Morrison, and J. Reis, *Proteasome protease mediated regulation of cytokine induction and inflammation*. Biochimica et biophysica acta, 2012. **1823**(11): p. 2087-2093.
109. Parks, W.C., C.L. Wilson, and Y.S. Lopez-Boado, *Matrix metalloproteinases as modulators of inflammation and innate immunity*. Nat Rev Immunol, 2004. **4**(8): p. 617-629.
110. Shaykhiev, R. and R.G. Crystal, *Innate Immunity and Chronic Obstructive Pulmonary Disease - A Mini-Review*. Gerontology, 2013. **59**(6): p. 10.1159/000354173.
111. Kay, A.B., *Immunosuppressive agents in chronic severe asthma*. Allergy proceedings : the official journal of regional and state allergy societies, 1994. **15**(3): p. 147-50.
112. Arend, W.P., M. Malyak, C.J. Guthridge, and C. Gabay, *Interleukin-1 receptor antagonist: role in biology*. Annual Review of Immunology, 1998. **16**(1): p. 27.
113. Peters, V.A., J.J. Joesting, and G.G. Freund, *IL-1 receptor 2 (IL-1R2) and its role in immune regulation*. Brain, behavior, and immunity, 2013. **32**: p. 1-8.
114. Park, W.Y., R.B. Goodman, K.P. Steinberg, J.T. Ruzinski, F. Radella, 2nd, D.R. Park, et al., *Cytokine balance in the lungs of patients with acute respiratory distress syndrome*. American journal of respiratory and critical care medicine, 2001. **164**(10 Pt 1): p. 1896-1903.
115. Zhang, H.Q., Y. Sun, and F. Xu, *Therapeutic effects of interleukin-1 receptor antagonist on allergic rhinitis of guinea pig*. Acta pharmacologica Sinica, 2003. **24**(3): p. 251-5.
116. Hallegua, D.S. and M.H. Weisman, *Potential therapeutic uses of interleukin 1 receptor antagonists in human diseases*. Annals of the Rheumatic Diseases, 2002. **61**(11): p. 960-967.
117. Ray, R., Z. Zhang, Y.C. Lee, J.L. Gao, and A.B. Mukherjee, *Uteroglobin suppresses allergen-induced TH2 differentiation by down-regulating the expression of serum amyloid A and SOCS-3 genes*. FEBS Lett, 2006. **580**(25): p. 6022-6.
118. Chiba, Y., R. Kurotani, T. Kusakabe, T. Miura, B.W. Link, M. Misawa, et al., *Uteroglobin-related Protein 1 Expression Suppresses Allergic Airway Inflammation in Mice*. American journal of respiratory and critical care medicine, 2006. **173**(9): p. 958-964.
119. Heikkinen, T., M. Shenoy, R.M. Goldblum, and T. Chonmaitree, *Quantification of cytokines and inflammatory mediators in samples of nasopharyngeal secretions with unknown dilution*. Pediatric research, 1999. **45**(2): p. 230-4.
120. Gabay, C. and I. Kushner, *Acute-phase proteins and other systemic responses to inflammation*. The New England journal of medicine, 1999. **340**(6): p. 448-54.
121. Kholodenko, B.N., *cell signalling dynamics in time and space*. Nature reviews. Molecular cell biology, 2006. **7**(3): p. 165-176.
122. Casado, B., L.K. Pannell, P. Iadarola, and J.N. Baraniuk, *Identification of human nasal mucous proteins using proteomics*. Proteomics, 2005. **5**(11): p. 2949-2959.
123. Ghafouri, B., B. Ståhlbom, C. Tagesson, and M. Lindahl, *Newly identified proteins in human nasal lavage fluid from non-smokers and smokers using two-dimensional gel electrophoresis and peptide mass fingerprinting*. PROTEOMICS, 2002. **2**(1): p. 112-120.

124. Wahlen, K., L. Fornander, P. Olausson, K. Ydreborg, U. Flodin, P. Graff, et al., *Protein profiles of nasal lavage fluid from individuals with work-related upper airway symptoms associated with moldy and damp buildings*. Indoor Air, 2016. **26**(5): p. 743-54.
125. Wang, H., J. Gottfries, F. Barrenäs, and M. Benson, *Identification of Novel Biomarkers in Seasonal Allergic Rhinitis by Combining Proteomic, Multivariate and Pathway Analysis*. PLoS ONE, 2011. **6**(8): p. e23563.
126. Tenzer, S., D. Docter, J. Kuharev, A. Musyanovych, V. Fetz, R. Hecht, et al., *Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology*. Nat Nano, 2013. **8**(10): p. 772-781.
127. Surinova, S., R. Schiess, R. Huttenhain, F. Cerciello, B. Wollscheid, and R. Aebersold, *On the development of plasma protein biomarkers*. J Proteome Res, 2011. **10**(1): p. 5-16.

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