



Q1 Differences in the coronal proteome acquired by particles depositing in the lungs of asthmatic versus healthy humans

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Abstract

Most inhaled nanomedicines in development are for the treatment of lung disease, yet little is known about their interaction with the respiratory tract lining fluids (RTLFs). Here we combined the use of nano-silica, as a protein concentrator, with label-free snapshot proteomics (LC-MS/MS; key findings confirmed by ELISA) to generate a quantitative profile of the RTLf proteome and provided insight into the evolved corona; information that may be used in future to improve drug targeting to the lungs by inhaled medicines. The asthmatic coronal proteome displayed a reduced contribution of surfactant proteins (SP-A and B) and a higher contribution of α 1-antitrypsin. Pathway analysis suggested that asthmatic RTLfs may also be deficient in proteins related to metal handling (e.g. lactoferrin). This study demonstrates how the composition of the corona acquired by inhaled nanoparticles is modified in asthma and suggests depressed mucosal immunity even in mild airway disease.

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Recent studies have shown how inhaled particle surfaces are modified when immersed in respiratory tract lining fluid (RTLf), becoming enriched with immune regulatory proteins, forming a corona that will be presented to the epithelium or phagocytic cells at the mucosal surface.^{1–4} This corona masks the original particle surface, changing its biological identity and potentially modifying the nature of the particles' interaction with the lungs, e.g. toxicity, cellular uptake and, in the case of nanomedicines,

therapeutic efficacy.² An understanding of these interactions is therefore essential for the development of inhaled medicines and yet in contrast to the abundance of published work on intestinal fluids and their interactions with oral dosage forms in the field of oral biopharmaceutics,⁵ little work has focused on particle interactions with RTLf components, especially in the context of target lung diseases.¹ In order to address this knowledge gap here we report the first visualization the corona formed in human

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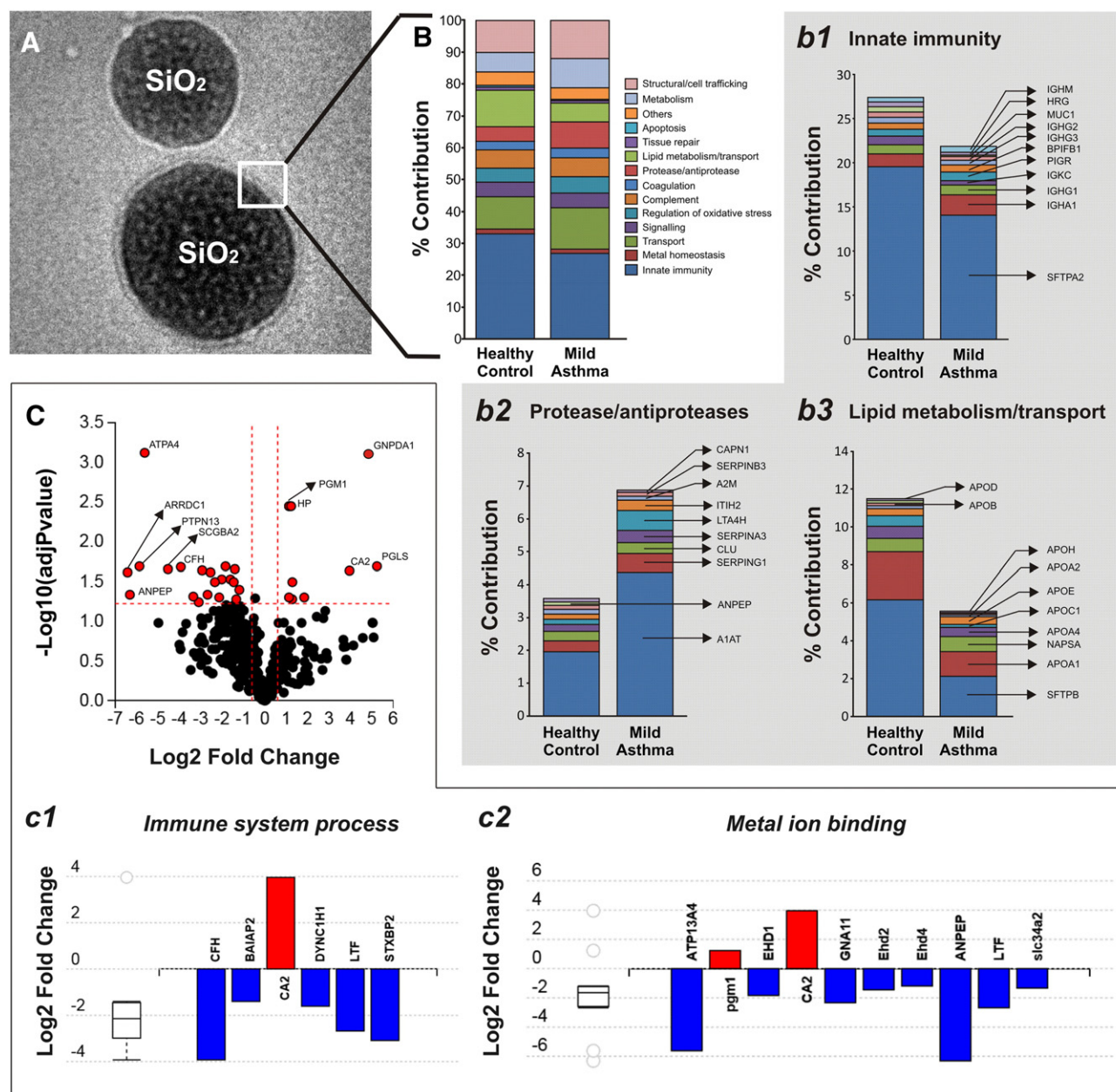


Figure 1. Proteomic characterization of the hard corona formed around SiO₂ particles incubated in concentrated bronchoalveolar lavage (BAL) fluids from healthy and mild asthmatic subjects. Panel A, cryoTEM image of SiO₂ particle incubated in concentrated healthy human BAL fluid (see Supplementary Information Figure 1). Panel B, functional classification and relative abundance of proteins identified in the healthy and asthmatic corona; panel *b1*, most abundant innate immunity proteins within the corona of the two groups (proteins are identified using their gene IDs); panel *b2*, most abundant proteins in the protease/antiprotease group; panel *b3*, relative contribution of proteins involved in lipid metabolism and transport. Panel C illustrates the results of the iPathway analysis, with proteins with significant over, or under representation in the asthmatic corona (relative to the healthy; see Supplementary Information Figure 2) highlighted using a volcano plot; panel *c1*, illustrates perturbation of proteins within the asthmatic corona related to immune system processes, using the conventional GO ontology; and panel *c2*, perturbations in proteins related to metal ion binding.

RTLF and provided a detailed characterization of the biomolecular corona of particles in healthy versus asthmatic RTLFs.

We studied the corona formed around silica nanoparticles (200 nm), a well characterized analytical standard,¹ incubated in concentrated bronchoalveolar lavage (BAL) fluids obtained from atopic asthmatics (PC₂₀ < 8 mg/mL methacholine, treated with short-acting inhaled β_2 -agonists on demand) and healthy control subjects. For these studies we employed lavage fluids from 5

healthy volunteers (26.0 $\pm$ 2 years, 2 M/3F) and 5 asthmatics (25 $\pm$ 6 years, 1 M/4F, PC₂₀ = 3.1 $\pm$ 2.6) concentrated back to 53 their undiluted RTLF levels based on the ratio of lavage to 54 plasma urea concentrations (a dilution factor to indicate the 55 degree to which RTLF has been diluted in the recovered BAL 56 fluid⁶), prior to pooling to provide a generic healthy and 57 asthmatic sample. Experimental details are outlined in the 58 Supplementary material. It was important to control for age as 59

Table 1

The 20 most abundant proteins (in rank order) based on the mean intensity of the three major precursor ions identified in the SiO₂ corona evolved after a 1-h incubation in concentrated lavage pooled from healthy and asthmatic subjects.

Top 20 abundant proteins in the SiO₂ corona

Healthy Controls			Mild asthmatics		
Identified Proteins	Relative rank in asthmatics	Molecular Weight	Identified Proteins	Relative rank in healthy controls	Molecular Weight
Pulmonary surfactant-associated protein A2	1	26 kDa	Pulmonary surfactant-associated protein A2	1	26 kDa
Serum albumin	2	69 kDa	Serum albumin	2	69 kDa
Pulmonary surfactant-associated protein B	6	42 kDa	Alpha-1-antitrypsin	6	47 kDa
Apolipoprotein A-I	9	31 kDa	Actin, cytoplasmic 1	5	42 kDa
Actin, cytoplasmic 1	4	42 kDa	Ig alpha-1 chain C region	8	38 kDa
Alpha-1-antitrypsin	3	47 kDa	Pulmonary surfactant-associated protein B	3	42 kDa
Complement C3	7	187 kDa	Complement C3	7	187 kDa
Ig alpha-1 chain C region	5	38 kDa	Complement C4-B	9	193 kDa
Complement C4-B	8	193 kDa	Apolipoprotein A-I	4	31 kDa
Hemoglobin subunit beta	12	16 kDa	Ig gamma-1 chain C region	11	36 kDa
Ig gamma-1 chain C region	10	36 kDa	Polymeric immunoglobulin receptor	14	83 kDa
Ig kappa chain C region	33	12 kDa	Hemoglobin subunit beta	10	16 kDa
Ubiquitin-60S ribosomal protein L40	63	15 kDa	Fibronectin	22	263 kDa
Polymeric immunoglobulin receptor	11	83 kDa	BPI fold-containing family B member 1	17	52 kDa
Annexin A2	32	39 kDa	Napsin-A	16	45 kDa
Napsin-A	15	45 kDa	Vitamin D-binding protein	56	53 kDa
BPI fold-containing family B member 1	14	52 kDa	Alpha-enolase	18	47 kDa
Alpha-enolase	17	47 kDa	Hemoglobin subunit alpha	45	15 kDa
Apolipoprotein A-IV	34	45 kDa	Ig mu chain C region	27	49 kDa
Ig gamma-3 chain C region	30	41 kDa	Glutathione S-transferase A2	94	26 kDa

For each protein in the healthy corona, the ranking in terms of abundance in the asthmatic group is provided (and vice versa). Albumin is highlighted as the signal reflects the residual contribution after pre-depletion of albumin (the most abundant protein in RTLTF) to enable identification of other proteins.

a factor because of the known age-related changes in the lung, reflected functionally by decreased lung volumes, reduced mucociliary clearance and increased susceptibility to infection.⁷

Cryogenic transmission electron microscopy was employed to provide a direct visualization of corona formation around nanoscale particles after incubation in human RTLTF from a healthy volunteer (Figure 1, A). Electron micrographs revealed a clearly visible corona, appearing as a continuous layer of electron-dense coating with a thickness of 3–5 nm surrounding the particles. This is in agreement with similar observations of corona formation around silica nanoparticles using Survanta®, an FDA approved bovine lung surfactant.¹ The albumin-depleted coronal proteome was characterized by label-free quantitative liquid chromatography mass spectrometry (LC–MS/MS) using established methods,¹ with over 400 coronal proteins identified, of which the most abundant 20 are highlighted in Table 1. For reasons of analytical sensitivity, it was necessary to deplete the lavage concentrates of albumin before incubation of the particles and proteomic characterization. Thus, the contribution of the most abundant RTLTF protein to the corona is artificially under-represented in the analysis. We did not address the lipid components of the corona, although these are thought to be less discriminating.^{3,4}

Identified proteins were functionally classified using SwissProt and NCBI databases (Figure 1, B), with the abundance of individual proteins expressed as the percentage of the intensity of all the proteins identified. Using this approach a ~10% decrease in the contribution of innate immunity proteins was identified in the asthmatic corona, with surfactant protein A (SP-A) the most dysregulated (0.47-fold decrease; Figure 1, Bi). A 5% decrease in the lipid metabolism/transport proteins was also observed in the asthmatic corona (Figure 1, Bii). Conversely, the asthmatic corona was associated with a 5% increase in proteins involved in protease-antiprotease activity (Figure 1, Biii), including a 1.7-fold increase of α 1-antitrypsin. These differences in the coronal proteome reflect differences reported previously in the composition of BAL fluid from asthmatics. Wu et al.⁸ and Cederfur et al.,⁹ using LC–MS/MS, also found that the majority of the differentially expressed proteins in asthmatic RTLTFs were associated with immune response (23%), lipid metabolism (12%) and proteolysis (9%). Thus, the coronal proteome appears to represent a concentrated fingerprint reflecting underlying protein composition of the host RTLTF.¹⁰ Differences between the corona formed in healthy and asthmatic RTLTF were evaluated further using iPathwayGuide analysis to obtain biological insights by identifying the pathways/functions most impacted by the observed changes between the two groups. This software analysis tool

Table 2

Selected protein concentrations and LPS activity quantified in bronchial wash and bronchoalveolar lavage fluid samples from expanded groups of healthy and asthmatic subjects.

	Fold change (Proteomic analysis)	Bronchial wash		Bronchoalveolar lavage	
		Healthy	Mild asthmatic	Healthy	Mild asthmatic
SP-A (mg/ml)	0.47	1.04 (0.60–1.54)	0.46 (0.23–0.70)	1.00 (0.61–1.31)	0.64 (0.46–0.87)
α 1AT (mg/ml)	1.74	0.68 (0.39–1.09)	0.38 (0.26–0.44)	0.94 (0.55–2.16)	1.55 (1.35–2.19)
IgA (mg/ml)	1.11	4.93 (1.80–7.76)	3.68 (0.67–4.92)	0.72 (0.53–0.97)	0.65 (0.28–1.24)
LPS (EU/ml) ^a	NA	4.1 (1.6–13.3)	27.1 (8.7–125.9)*	14.3 (5.5–40.4)	28.3 (9.2–76.0)

Data represent median values with the 25th and 75th percentiles, $n = 14$ –15 per group, reflecting the availability of lavage material.

All protein concentrations and LPS activities have been adjusted for sample dilution based on the ratio of plasma to lavage urea concentrations. Comparison of healthy and mild asthmatic lavage protein concentrations was performed using the Mann-Whitney U test, with significance assumed at the 5% level.

^a Lipopolysaccharide activities were determined using Limulus Amebocyte Lysate assay and reported in EU/ml.

implements an ‘impact analysis’ approach, which takes into consideration not only the number of differentially expressed genes, or in this case proteins (i.e. enrichment analysis), but also topological information such as the direction and type of all signals in a pathway, and the position, role, and type of each protein.¹¹ Following quantile normalization and Benjamini-Hochberg correction for false discovery rate, we identified 26 differentially adhered proteins in the asthmatic corona (Figure 1, C). Pathway analysis of these proteins demonstrated that they were involved in immune system processes (Figure 1, Ci) and metal ion binding (Figure 1, Cii), with lactoferrin contributing to both pathways.

To evaluate the accuracy of this semi-quantification of coronal protein content, selected proteins were quantified by ELISA in lavage samples, both bronchial wash (BW) and BAL fluid from an expanded group of asthmatics (26.0 ± 6 years, 6 M/10F, $PC_{20} = 2.5 \pm 2.6$) and healthy (25 ± 2 years, 11 M/5F) subjects. Three proteins were examined: Immunoglobulin A (IgA, the major mucosal immunoglobulin, the levels of which appeared equivalent in the proteomic analysis), surfactant protein A (SPA, 0.47-fold decreased in asthmatics) and alpha-1-antitrypsin (α 1AT, 1.74 fold increased in asthmatics) (Table 2). Although the differences in protein concentrations as determined by ELISA analysis did not attain statistical significance, the observed trends for reduced SP-A ($P = 0.06$) and increased alpha-1-antitrypsin were consistent with the proteomic analysis. We extended the validation panel to include five further proteins, 4 selected of the basis of their high abundance within the coronal proteome (albumin, IgG, IgM and transferrin), and the fifth due to the evidence of suppressed innate immune defense proteins (lysozyme). These data are summarized in Supplementary Table 1, together with the predicted fold changes obtained from the proteomic analysis. Of these proteins both transferrin and lysozyme appeared to be most depressed within the mild asthmatic corona, 0.16 and 0.34-fold respectively. The more comprehensive ELISA data within the BAL fluid did not confirm this finding, but a significant ($P < 0.05$) decrease in transferrin within the asthmatic BW samples was observed, with a trend ($P = 0.06$) toward decreased lysozyme concentrations also within the proximal airway sample.

Whilst the proteomic and ELISA results were not wholly concordant, these data overall, together with the pathway analysis, could be suggestive of depressed innate immune defense in mild asthmatics. Since the deficiencies in immune

proteins identified within the asthmatic RTLF may increase susceptibility to microbial infection,¹² lipopolysaccharide (LPS) levels were quantified to provide a proxy measure of bacterial load within the airway (see Supplementary material). BAL fluid LPS activity was found to be 1.98-fold higher in asthmatics compared to healthy subjects, but did not attain statistical significance (Table 2). In contrast, a significant 6.6-fold increase in LPS was noted in the more proximal BW sample from the asthmatics ($P < 0.05$).

These data provide a detailed molecular characterization of the asthmatic coronal proteome, with evidence of deficiencies in proteins involved in innate immunity, lipid metabolism/transport and metal handling. Our observations may raise concerns about the impact of proposed therapeutic inhaled nanomedicines on innate immunity in the lung. The mechanism we have employed here to concentrate RTLF proteins, for characterization purposes, may in vivo result in an unwanted sequestration of RTLF proteins. Axiomatically, the adsorption of proteins that trigger phagocytosis of a particle provides a clearance-promoting mechanism. SP-A binding has been shown to trigger the uptake of particles in murine alveolar macrophages,^{13,14} thus its reduced presence in the asthmatic corona may impair bacterial clearance. Similarly, the formation of the particle corona may lead to local depletion of innate immunity proteins at the surface of the lungs. Thus whilst the formation of the corona may modify particle clearance^{15–17} thereby affecting drug disposition in the lungs, it may also increase the susceptibility of the airway to infection. Consistent with this, exposure to ultrafine carbon black particles has been shown to increase the susceptibility of the lung to both bacterial¹⁸ and viral infections¹⁹ in vivo. Susceptibility to infection has also been reported in populations exposed to cigarette smoke²⁰ combustion-derived air pollution²¹ and occupational aerosols.²² This therefore raises the question as to whether inhaled slowly disintegrating nanoscale drug formulations may have adverse effects on immunity in the lung, especially with regular use. Such considerations do not apply to current inhaled medicines which exist transiently, if at all, in solid particle form in RTLF, although interestingly studies have demonstrated an increased risk of pneumonia in COPD patients using poorly soluble inhaled corticosteroids.^{23,24} It is clearly important to understand particle–RTLF interactions and further work is needed in this area focused on relevant patient groups.

Appendix A. Supplementary data

Supplementary data to this article can be found online at
<http://dx.doi.org/10.1016/j.nano.2017.06.008>.

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Supplementary material

Materials and methods

Human respiratory tract lining fluid

Lavage was performed on healthy ($n=5$, 26 ± 2 years, 2M/3F) and 5 mild atopic asthmatics (24.6 ± 6.2 years, 1M/4F, $PC_{20}=3.07\pm 2.63$) subjects following an overnight fast as previously described (1, 2). Briefly, an initial bronchial wash was performed either in the right middle lobe or the left lingula lobe by the instillation and immediate aspiration of 2×20 mL of sterile saline (37°C). These initial samples of the conducting airway RTLFs are not reported on in this paper, as the recovered volumes were insufficient for processing. Following the bronchial wash, a larger volume bronchoalveolar lavage (BAL, 3×60 mL) was performed at the same site. The aspirates recovered from each of the 60 mL instillations were collected and pooled into a siliconised container on ice. All lavage samples were filtered through nylon (pore diameter $100\text{ }\mu\text{m}$) and centrifuged at 400 g for 15 min to isolate the cell free supernatant. The supernatants were then stored at -80°C until required for further processing. Sample concentration, following sample thawing, was performed using 9K MWCO iCON Pierce concentrators (Thermo Scientific), by centrifugation at $4,000\text{ rpm}$ at 4°C , for 15 min cycles until complete. The filter retentate was resuspended in 1 mL HBSS and the protein concentration of the five individual concentrated BAL samples (reconstituted RTLF), as well as a pooled sample derived from equal volumes ($200\text{ }\mu\text{L}$) of the separate samples were determined using the bicinchoninic acid assay, as previously described. The highly abundant plasma protein albumin was depleted from all the reconstituted RTLF samples, using SwellGel Blue Albumin Removal Discs (Pierce), according to the manufacturer's instructions to enhance the quantification of low abundance proteins using downstream proteomic approaches. Briefly, samples were loaded onto a single removal disc, where each disc is reported to have a binding capacity of 2 mg of albumin and incubated for 2 minutes prior to centrifugation at $12,000\text{ g}$ for 1 minute . The flow-through was reapplied to the disc and incubated for a further 2 minutes to ensure maximal binding prior to centrifugation at $12,000\text{g}$ for 1 minute . The unbound protein from the disc was released by adding $50\text{ }\mu\text{L}$ of wash buffer provided and centrifugation for 1 minute . It should be noted therefore that the concentrated BAL fluid is not should a fully reconstituted RTLF because of the necessity to pre-deplete albumin. This approach is commonly applied in proteomic studies (3) and trades the underestimation of the 'true' contribution of albumin, and potentially the loss of constituents below the molecular weight cut-off, for the ability to quantify low abundance proteins. Informed written consent was obtained from all subjects prior to inclusion into this study, which was approved by the Regional Ethical Review Board at Umeå University, Sweden and performed, in accordance with the Declaration of Helsinki.

Physicochemical characterisation

SiO₂ nanoparticles (200 nm) were purchased from Kisker Biotech GmbH & Co. KG (Steinfurt, Germany). Particle size and morphology were characterised in both buffer and the pooled concentrated human lung lining fluid using cryo-transmission electron microscopy (cryo-TEM). The cryo-TEM measurements were carried out using a Zeiss Libra 120 Transmission Electron Microscope (Carl Zeiss NTS, Oberkochen, Germany). Analyses were performed under cryo-conditions, with the microscope operating at 80 kV in zero loss bright-field mode. Digital images were recorded under low dose conditions, with a slow-scan CCD camera (TRS GmbH, Moorenweis, Germany) and iTEM software (Olympus Soft Imaging System GmbH, Münster, Germany). An under focus of 1-2 μm was used to enhance the image contrast.

Prior to imaging, the samples were treated by means of placing a drop of sample solution on a copper grid covered with a carbon reinforced holey polymer film (hole size $\sim 2\text{--}6\ \mu\text{m}$) and then thinned by blotting with filter paper. This was done in a custom-built climate chamber at 25°C and close to 100% relative humidity in order to avoid dehydration of the samples. Once blotted the samples were quickly vitrified in liquid ethane held at a temperature just above its freezing point (-182 °C). After vitrification the samples were transferred to the microscope using a Gatan CT3500 cryo-transfer (Gatan, Oxon, UK), keeping samples below -165°C and protected against atmospheric conditions. The cryo-TEM technique is described in Almgren *et al* (4).

Dynamic light scattering was performed using Zetasizer Nano (Malvern Instruments) at $\theta = 173^\circ$. Briefly, particle stock was sonicated (2 min) prior to dilution of particles to 73 $\mu\text{g/mL}$ in either PBS or RTLF, which was immediately used for particle sizing at $t=0$ h. Following this, the particle sample in RTLF was incubated at 4°C for 1 h, prior to isolation of hard corona as described in previous section and sized using DLS. All measurements were performed at 25°C and values reported represent an average of six repetitions of one minute each and repeated three times. Data analysis has been performed according to standard procedures, and interpreted through a cumulant expansion of the field autocorrelation function to the second order.

Corona formation and isolation of coronal proteins

The isolation procedure of coronal proteins from the nanoparticle surface was performed as described previously (2). Samples of SiO₂ nanoparticles ($n=3$) were then incubated within the albumin-depleted, pooled, reconstituted human bronchoalveolar lavage fluid (as a model of undiluted RTLF) at a concentration matching the surface area to protein ratio ($1.9 \times 10^9\ \mu\text{m}^2/\text{mg}$), as previously used by Monopoli *et al.*(5) (particle concentration 73.5 $\mu\text{g/mL}$ in RTLF). Particle suspensions were incubated on ice for one hour to prevent the action of proteolytic enzymes present in the human RTLF. The samples

were then centrifuged for 1 h at 20,000 *g* (4°C) to pellet the particle-protein complexes and the supernatant was removed. The pellet was resuspended in 500 µL phosphate buffered saline (PBS) and centrifuged again for 1 h at 20,000 *g* (4°C). The washing procedure was repeated twice before resuspension of the final pellet in 14 µL PBS. The resuspended protein pellets were mixed with 7 µL protein loading buffer [62.5 mM Tris-HCL pH 6.8, 2% (w/v) SDS, 10% glycerol, 0.04M DTT and 0.01% (w/v) bromophenol blue] and heated for 5 min at 100° C. The entire sample volume was loaded onto a 12% polyacrylamide gel and electrophoresis was performed at 120 V, 400 mA. The samples were placed onto the stacking gel and allowed to run until all the sample had migrated to the separating gel. The run was stopped before the separation of the proteins in the stacking gel. Gels were stained for 1 h in Coomassie blue stain [50% methanol, 10% acetic acid, 2.5% (w/v) brilliant blue, in water].

Protein identification

The entire band from the separation layer of the SDS-PAGE gel was excised and digested in-gel with trypsin according to the method of Shevchenko et al (6). The digested peptides were then resuspended in 0.1% formic acid. Electrospray liquid chromatography mass spectrometry (LC-MS/MS) equipped with a HPLC (Surveyor, ThermoFinnigan, CA) and interfaced with a Q-Exactive (ThermoFinnigan, CA) was used to analyze peptide mixtures following trypsin digestion.

Raw mass spectra were processed using Proteome Discoverer (version 1.4; Thermo Fisher Scientific Inc.) and searched against the latest release of the Uniprot database using the Mascot search algorithm (version 2.2; Matrix Science, London, UK). Mascot.dat files were uploaded into Scaffold (version Scaffold_3.6.3, Proteome Software Inc., Portland, OR, USA), which was used to validate MS/MS-based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95.0% probability, as specified by the Peptide Prophet algorithm (7). Protein identifications were accepted if they could be established at greater than 99.9% probability and contained at least 4 unique identified peptides. The abundance of an individual protein was calculated as the sum of the three most intense peptide precursor ions derived from that protein.

Protein classification and bioinformatics analysis

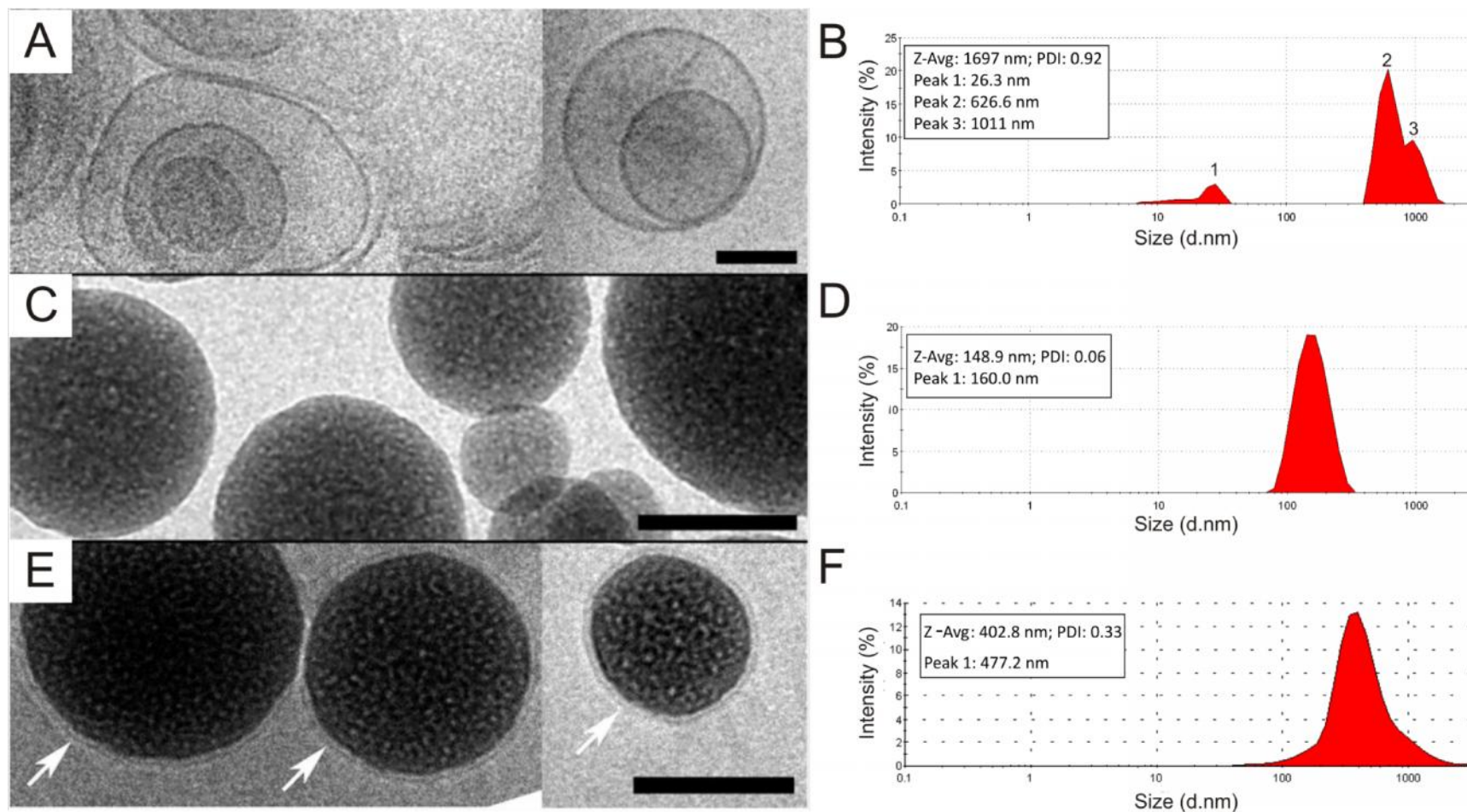
Prior to quantitative analysis quantile normalization of the proteomics dataset was performed (8) to remove systemic bias. The mean value of intensities from three experimental replicates was calculated for each protein after quantile normalization. The relative abundance of protein *k* in the corona was calculated by applying the following equation:

$$\% \text{ contribution of protein}_k = \frac{\text{Mean Intensity}_k}{\sum_{i=1}^N \text{Mean Intensity}_i}$$

Proteins were classified according to their known biological function in the lung using SwissProt and NCBI databases. One-way ANOVA was performed between the coronal proteins identified in the mild asthmatic RTLf and healthy RTLf using Qlucore Omics Explorer (Sweden). Benjamini–Hochberg correction was used to correct for the false discovery rate. Qlucore was also used to construct the heat map of the coronal proteins identified. Log₂ ratio of proteins (abundance in mild asthmatic relative to healthy RTLf) and false discovery rate corrected p-values were obtained from Qlucore. Gene name (obtained from Scaffold) for each protein along with log₂ ratio and p-values (from Qlucore) were used to perform pathway analysis of significantly different proteins using iPathwayGuide (Advaita, USA).

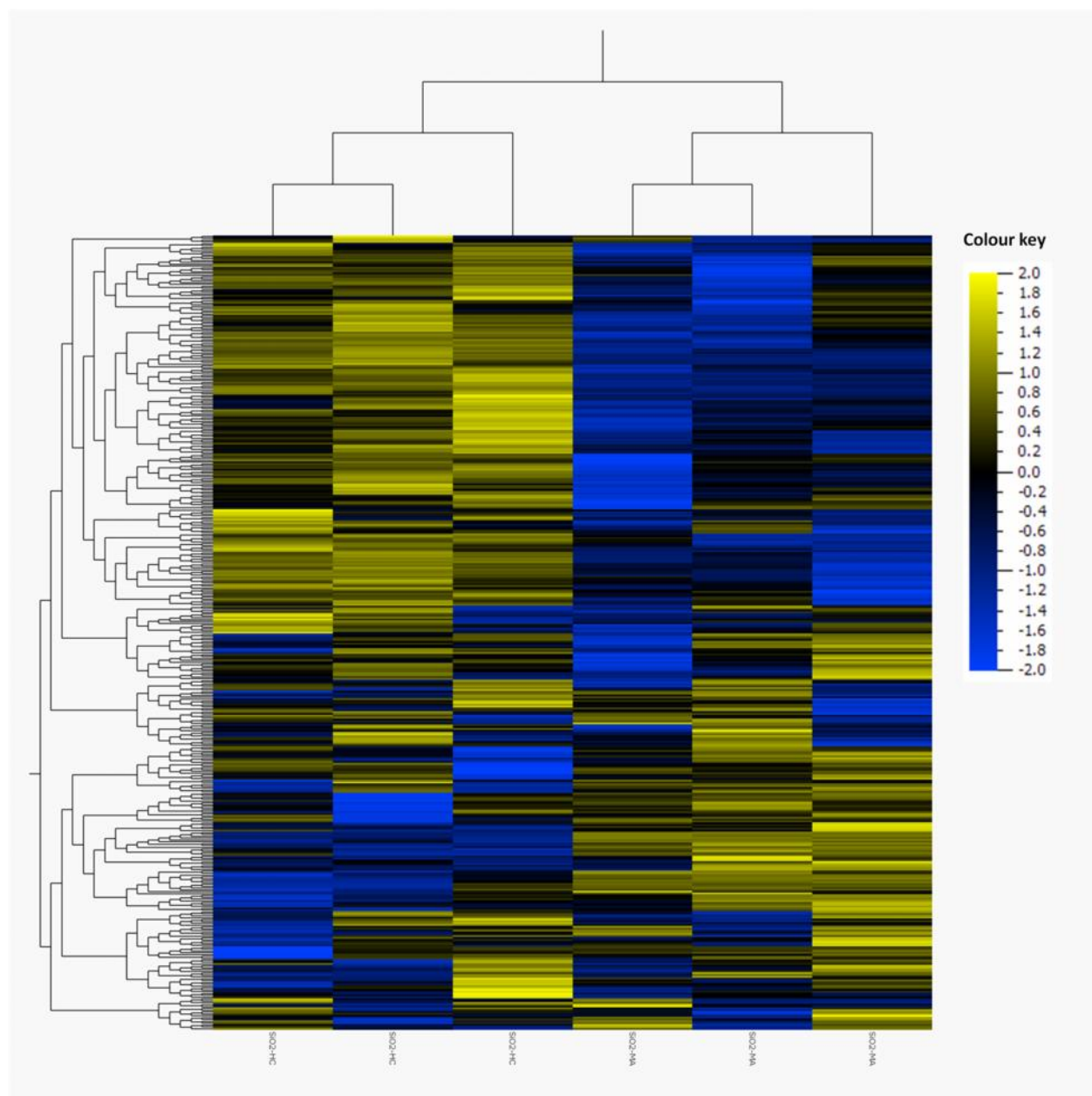
Validation by individual antibody based quantification and determination of bacterial load

BAL-fluid and BW samples were assayed for albumin using the bromocresol green assay (Alpha Diagnostics, San Antonio, Texas, USA). Enzyme-Linked Immunosorbent Assays (ELISA) were employed for all other protein analyses using the following commercially available kits: SP-A (Biovendor, Brno, Czech Republic), transferrin (Alpha Diagnostic International, San Antonio, Texas, USA) and lysozyme (Immunodiagnostik, Bensheim, Germany). All remaining proteins: α -1-AT, IgA, IgG and IgM, were analysed using ELISA kits purchased from Bethyl Laboratories (Texas, USA). BAL fluid LPS concentrations were determined using the kinetic Limulus Amebocyte Lysate (LAL) assay from Lonza (Walkersville, MD, USA). All analyses were performed according to manufacturer's instructions.



Supplementary Figure 1. Visualisation and characterisation of microstructures in human RTLFL and nanoparticle protein corona.

Microstructures imaged in the suspension of concentrated human RTLFL showing the presence of bilamellar and oligolamellar liposomal vesicles (A) scale bar: 100nm; silica nanoparticles appear as spheres of uneven density and with a rough surface (C) and silica nanoparticles after incubation with human RTLFL for 1 h appear surrounded by a ring of electron dense material, which corresponds to a lipid layer (biomolecular corona) covering the particle surface (E); scale bar: 100nm. Hydrodynamic diameters of nanostructures formed in RTLFL as determined by DLS (B), hydrodynamic diameters of SiO₂ nanoparticles in PBS (D) and the hydrodynamic diameters of SiO₂ nanoparticles after isolation of hard corona (F).



Supplementary Figure 2: Unsupervised hierarchical cluster analysis of the relative abundance of coronal proteins bound to SiO₂ nanoparticle in healthy RTL and mild asthmatic RTL. Color scheme is based on $\log_2$ of the ratio between mild asthmatic and healthy RTL coronal proteins. Yellow indicates proteins with higher than average abundance, blue indicates proteins with lower than average abundance.

Supplementary Table 1: Concentrations of selected proteins, in bronchial wash (BW) and bronchoalveolar lavage (BAL) fluid obtained from expanded groups of young healthy and mild atopic subjects. Data represent median values, with 25th and 75th percentiles, n =14-15, reflecting the availability of lavage material.

Protein (mg/ml)*	Fold change (Proteomic analysis) ^A	Bronchial wash		Bronchoalveolar lavage	
		Healthy	Mild asthmatic	Healthy	Mild asthmatic
Albumin	0.86 ^B	24.1 (18.2-30.0)	22.0 (14.7-30.6)	8.16 (6.49-9.37)	7.77 (5.34-10.04)
Transferrin	0.16	1.95 (1.29-3.06)	0.80 (0.54-1.53)*	1.09 (0.62-1.70)	1.51 (1.19-1.95)
Lysozyme	0.34	0.25 (0.12-0.42)	0.14 (0.04-0.16)	0.03 (0.02-0.06)	0.05 (0.02-0.08)
Ig G	0.52-0.71 ^C	2.33 (0.94-2.91)	1.75 (1.39-2.92)	1.59 (1.04-2.74)	2.13 (1.55-2.91)
Ig M	0.86	0.05 (0.02-0.14)	0.05 (0.03-0.41)	0.06 (0.04-0.18)	0.05 (0.03-0.09)

All concentrations have been adjusted for sample dilution based on the ratio of plasma to lavage urea concentrations. Comparison of healthy and mild asthmatic lavage protein concentrations was performed using the Mann-Whitney U test, with significance assumed at the 5% level (). **A:** all fold changes based on the data from the pooled RTLTF samples, as described in the main paper. **B:** fold change based on the albumin depleted proteome, BW and BAL fluid albumin concentrations. The validation analysis in the expanded groups was performed on non-depleted samples. **C:** the range of fold changes reflects the differences observed in the following identified proteins: Ig gamma-1 chain C region, Ig gamma-2 chain C region and the Ig gamma-3 chain C region.

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