Proteome analysis in ambient coarse and fine particles

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Metaproteomics has been applied in a variety of environments, e.g. marine, soil and sediment (Williams et al. 2010, Bruneel et al. 2011, Wang et al. 2014). However, to our knowledge, there is still a lack of studies analyzing the metaproteome of aerosols, although proteins account for up to 5% of urban air particulate matter, influence the physicochemical properties of atmospheric particles, and play a major role as airborne allergens (Franze et al. 2005). Characterization of the metaproteome in ambient particles will yield information about the atmospheric transformation of proteins, e.g. nitration, oxidation and degradation. Nitrated Bet v 1, the major birch pollen allergen, has an enhanced immunogenic effect compared to its non-nitrated variant (Ackaert et al. 2014). Therefore, a new method was developed to investigate the aerosol metaproteome in this study.

We developed an extraction method in which a high recovery was obtained for proteins from particles sampled on glass fiber filters (Fig 1). Sodium dodecyl sulphate (SDS), as an anionic detergent, is a powerful extractant and facilitates the solubilization of otherwise water-insoluble proteins as well as water-soluble proteins. Further, the foams produced by SDS buffer solutions during sonication or shaking could penetrate into the filter material and overcome the interaction of proteins with the filter material.

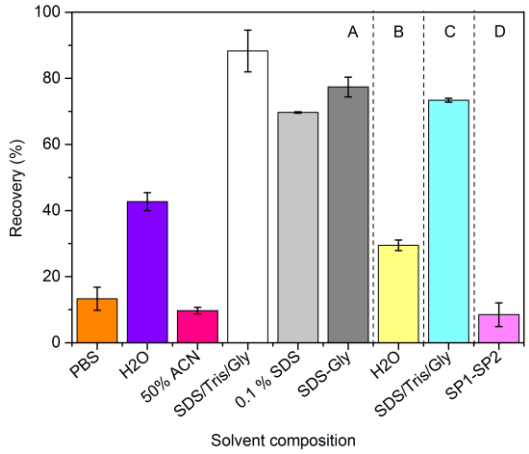

Figure 1. BSA recoveries from spiked filters samples using BCA assay. Extraction-Enrichment method: (A) sonication-freeze drying, (B) stirring-freeze drying, (C) shaking ground filter-freeze drying, (D) bead beating-precipitation.

Protein extracts were further cleaned up by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), which was used to remove the low molecular weight sample matrix and to spread out the proteome. SDS-PAGE lanes were divided into five fractions and excised into pieces for in-gel digestion according to a protocol by Shevchenko et al. (2007). Peptide mixtures were analyzed with a Thermo Q Exactive Plus Orbitrap mass spectrometer coupled to an EASY nLC 1000 uHPLC system.

To assess the suitability of the method for the analysis of proteomes in ambient particles, it was applied to filter samples from an urban/rural influenced sampling site in central Europe. Our results showed proteins from fungi, plants, bacteria and animals were the major components both in coarse (PM_{10}) and fine ($PM_{2.5}$) mode particles, which is in line with the major categories of primary biological aerosol particles (Després et al. 2012). Allergenic proteins were found both in PM_{10} and $PM_{2.5}$, suggesting allergens are able to be transported deep into the respiratory tract by fine mode aerosols. In addition, the observation of high molecular weight proteins in the low molecular weight SDS-PAGE fraction indicates a potential degradation process in the atmosphere.

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