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Detection of metabolite corona on amino functionalised polystyrene nanoparticles and its implications in freshwater organisms

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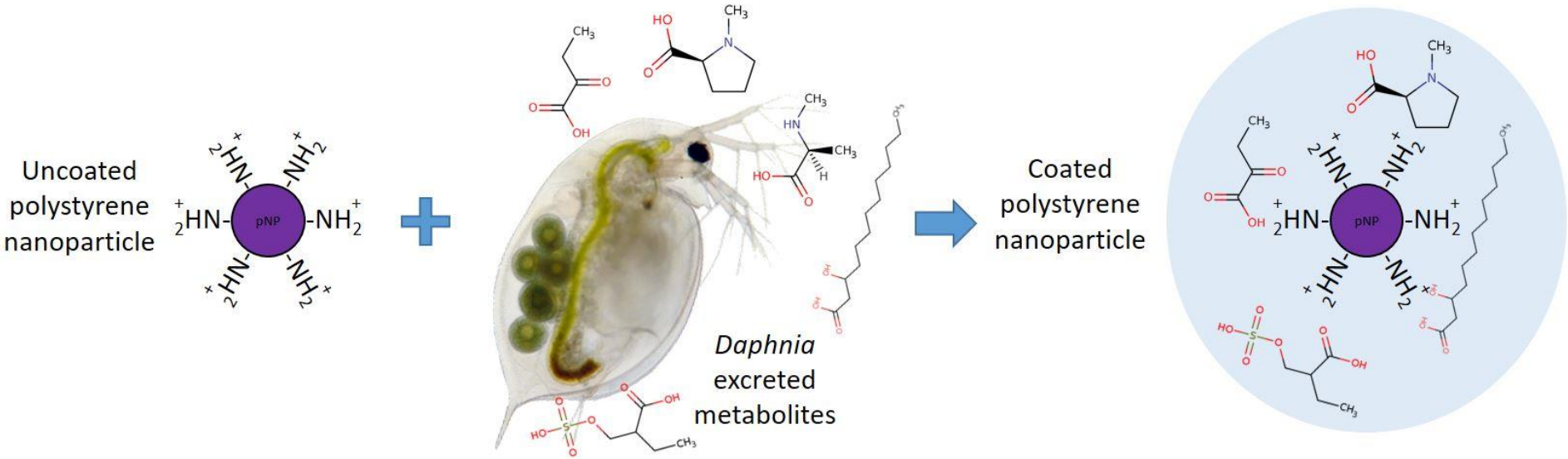
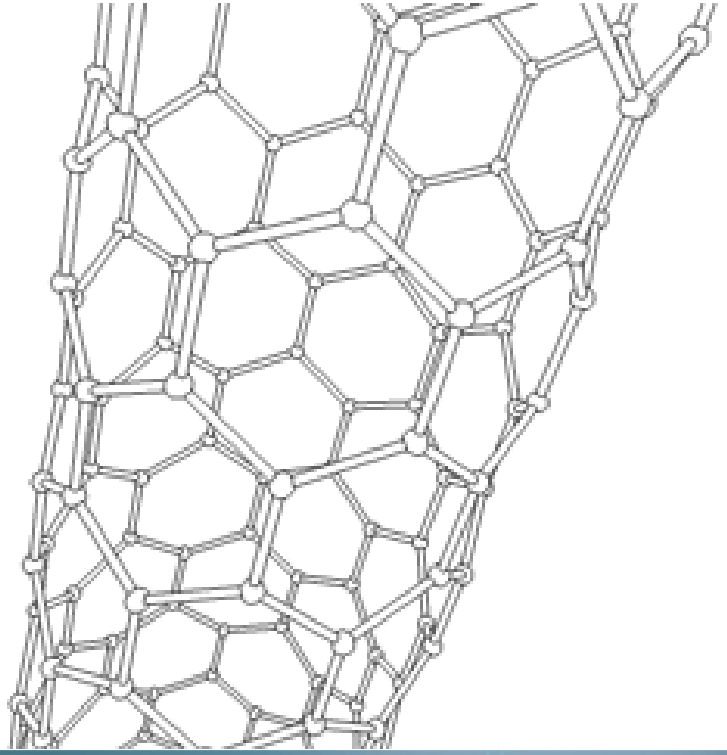


Abstract: Protein corona formation on nanoparticles (NPs), affect NP physicochemical properties, cellular uptake and toxicity, and has been reported extensively. To date, studies of the occurrence and potential importance of small molecule (metabolite) coronas are limited. We sought to determine such a corona using high-sensitivity metabolomics combined with a well-established model system for freshwater ecotoxicology (*Daphnia magna* feeding on *Chlorella vulgaris*) and amino functionalised polystyrene NPs (NH_2-pNPs). Initially, we optimised our method using a targeted LC-MS/MS approach for sodium dodecylsulphate (SDS) as an analogue to signalling molecules that are known to occur in our freshwater model system. Following, we performed an untargeted discovery metabolomics study – using high-sensitivity nanoelectrospray direct infusion mass spectrometry (DIMS) for the unbiased assessment of the metabolite corona of NH_2-pNPs in the freshwater model system. Untargeted DIMS metabolomics reproducibly detected 100s of small molecule peaks extracted from the NH_2-pNPs when exposed to conditioned media from the *D. magna-C. vulgaris* model system. Attempts to annotate these extracted metabolites, including through the application of van Krevelen and Kendrick Mass Defect plots, indicate a diverse range of metabolites that were not clustered into any particular class. Overall, we demonstrate the existence of an ecologically relevant metabolite corona on the surface of NPs through application of a high-sensitivity, untargeted mass spectrometry metabolomics workflow.

Keywords: metabolite corona, *Daphnia magna*, direct infusion mass spectrometry, untargeted metabolomics, polystyrene nanoparticles

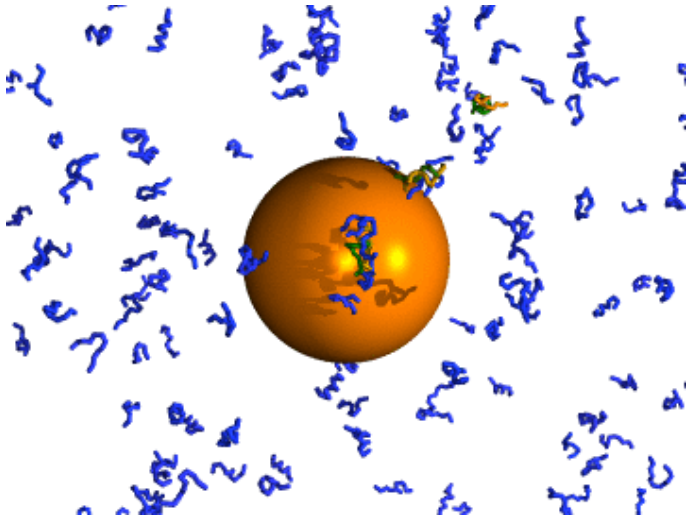
Introduction

- Engineered nanomaterials are currently being used in a wide range of commercial products due to their physico-chemical properties. As a result, their levels within the environment are increasing and this has raised concerns over the exposure and possible health effects on humans and other organisms.



Introduction

- Nanoparticles rapidly adsorb proteins on their surface in a competitive manner, generating a corona which affects their interactions with cells and organisms and in turn impacts their toxicity and uptake.
- So far, studies on NP coronas have focused on the protein component and even though the scientific community and literature anticipate that smaller molecules could also bind to nanoparticles, this novel component of the corona has not yet been fully characterised.
- The overall aim of the present study was to develop, optimise and apply a robust procedure for isolating the metabolites bound to positively charged amino functionalised polystyrene nanoparticles (NH_2-pNPs) and study its role in a freshwater system.

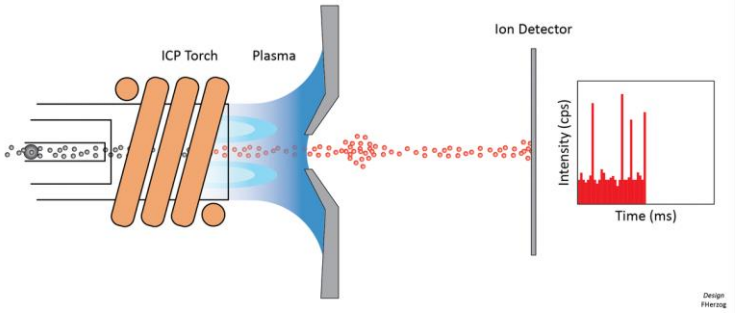


Introduction

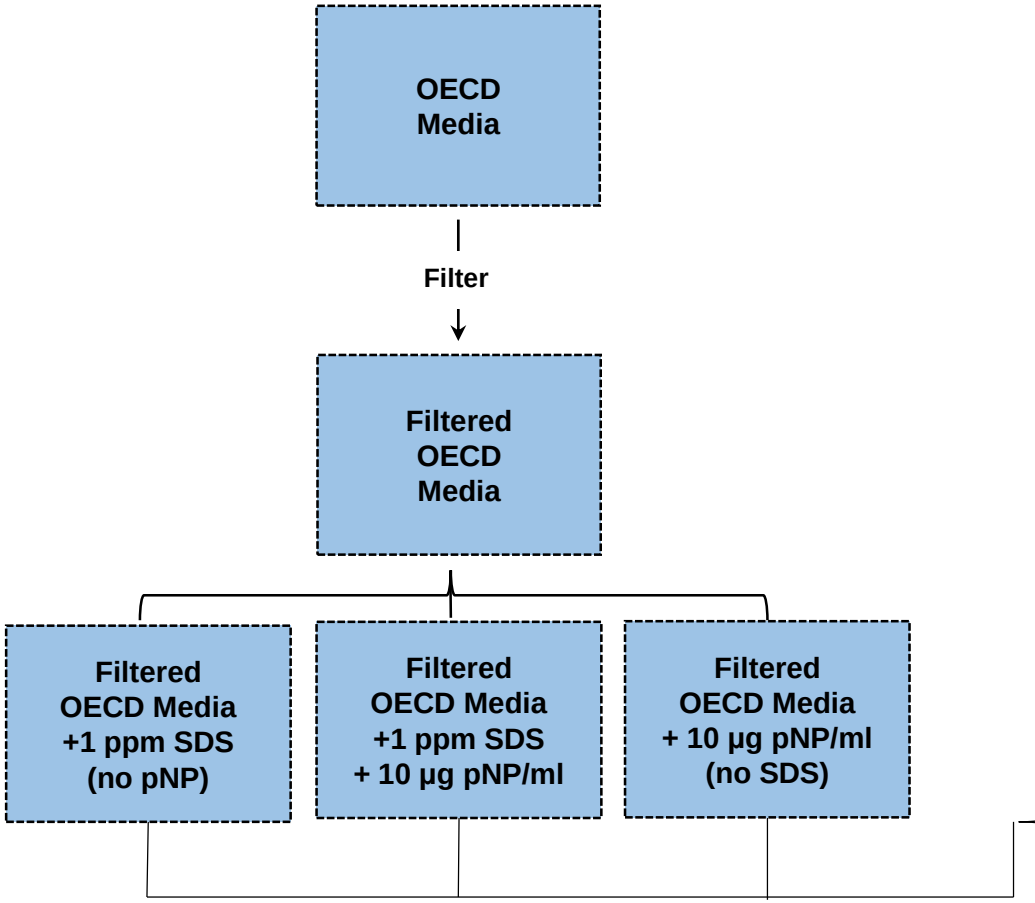
- Our first objective was to develop a protocol to isolate and measure this small molecule corona from NH_2-pNPs. We used sodium dodecyl sulphate (SDS) as a reference compound, due to its molecular similarity to sulphonated lipid chemical signalling molecules (kairomones) that are known to be excreted into the environment by *Daphnia*.

- The second objective was to expand beyond this targeted detection to a high-sensitivity untargeted metabolomics semi-quantitative method (via nanoelectrospray direct infusion mass spectrometry, DIMS) to detect metabolites excreted from a well-established model system for freshwater ecotoxicology that form a small molecule corona on NPs.

- Finally, using feeding performance we explored the impact of corona formation on *Daphnia magna* physiology.

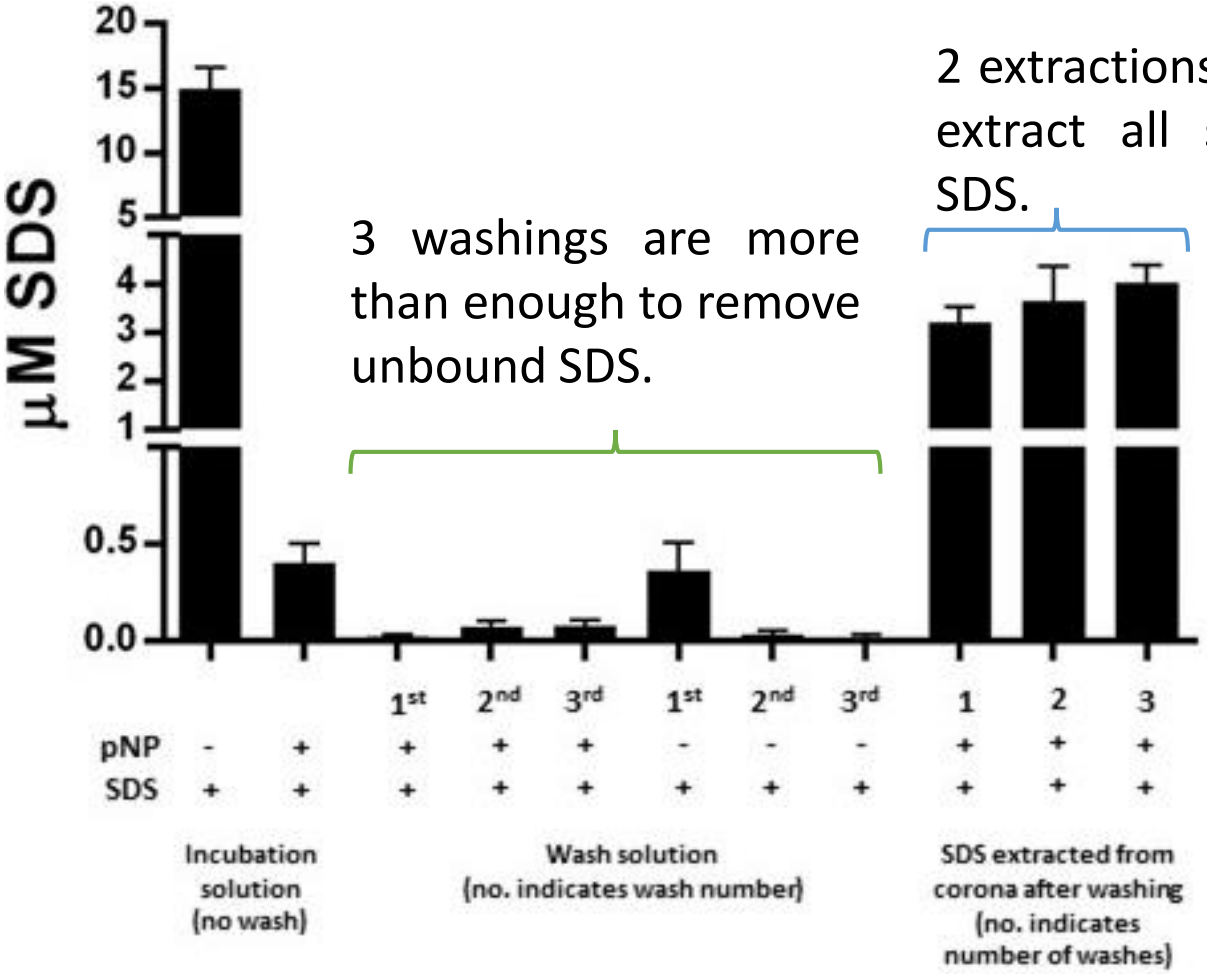


Results and Discussion: SDS optimisation



Wash steps
Extraction
Re-suspension
LC-MS/MS detection of SDS

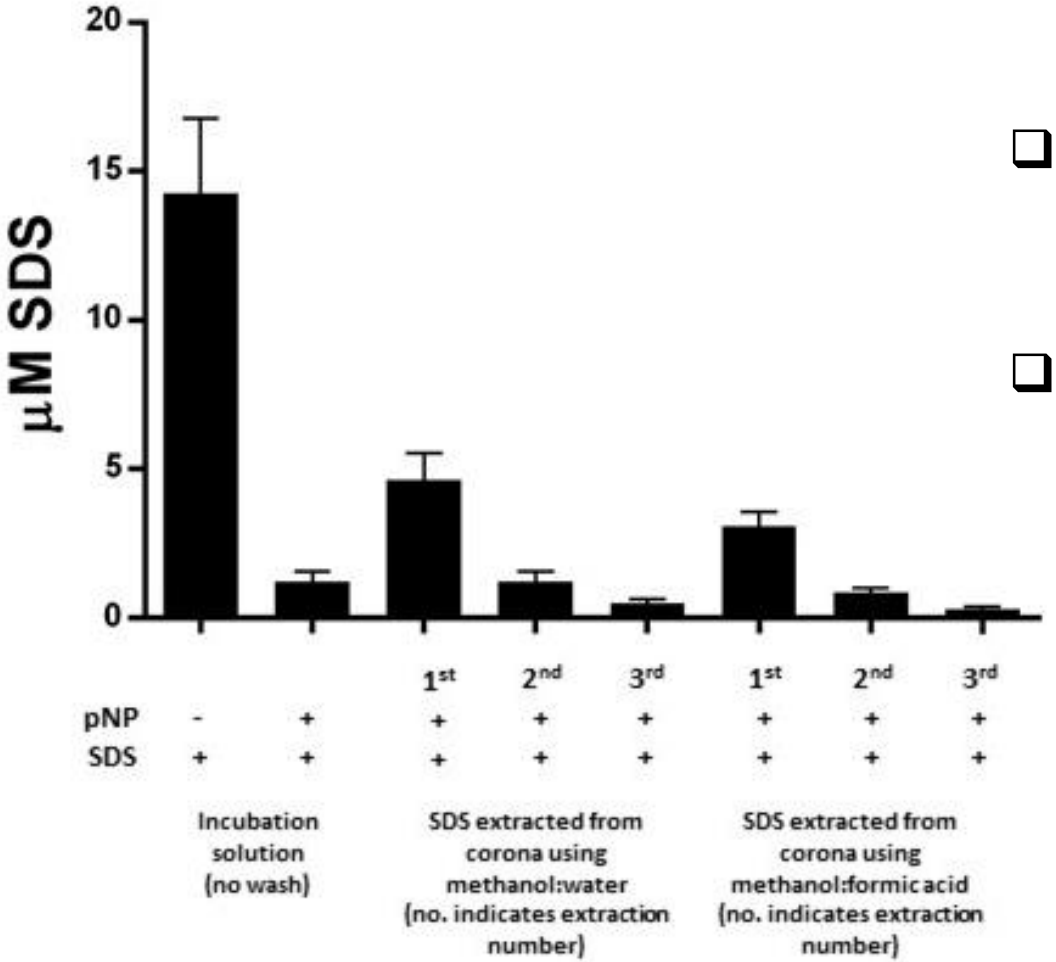
Results and Discussion: SDS optimisation I



3 washings are more than enough to remove unbound SDS.

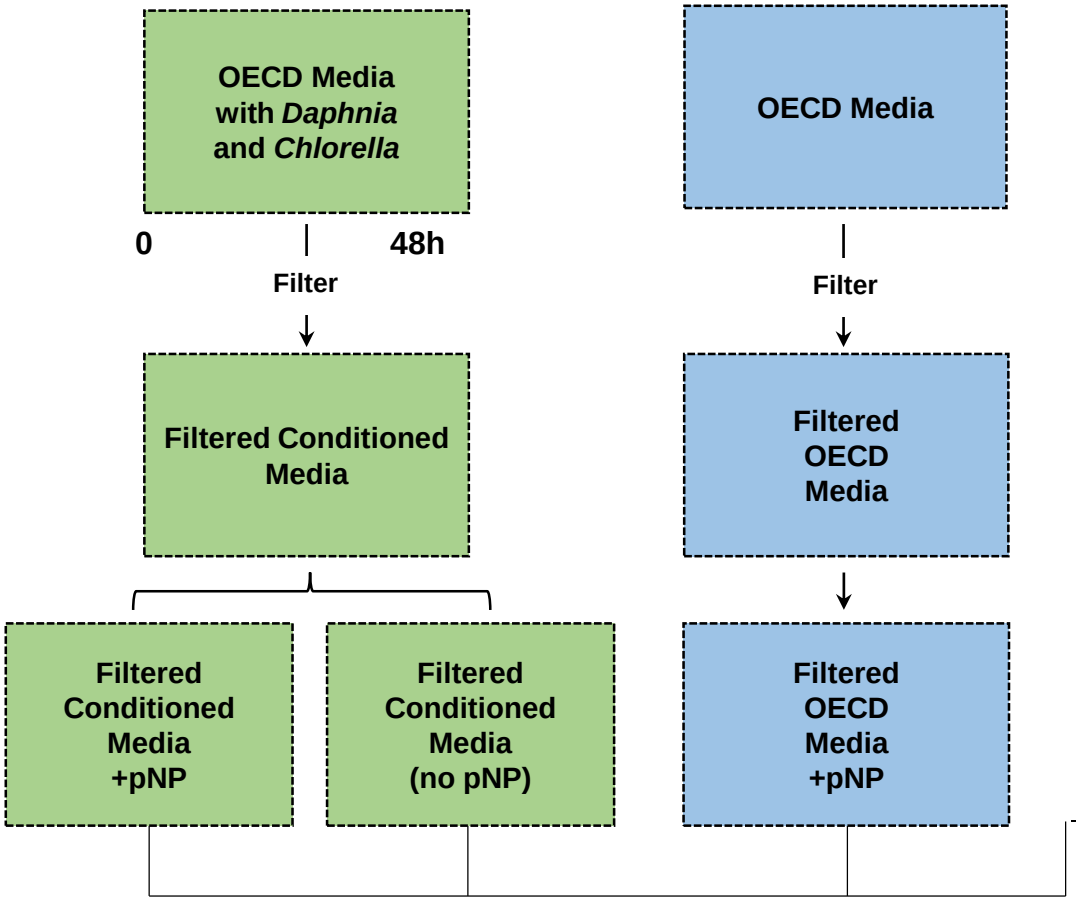
2 extractions are sufficient to extract all signal of bound SDS.

Results and Discussion: SDS optimisation II



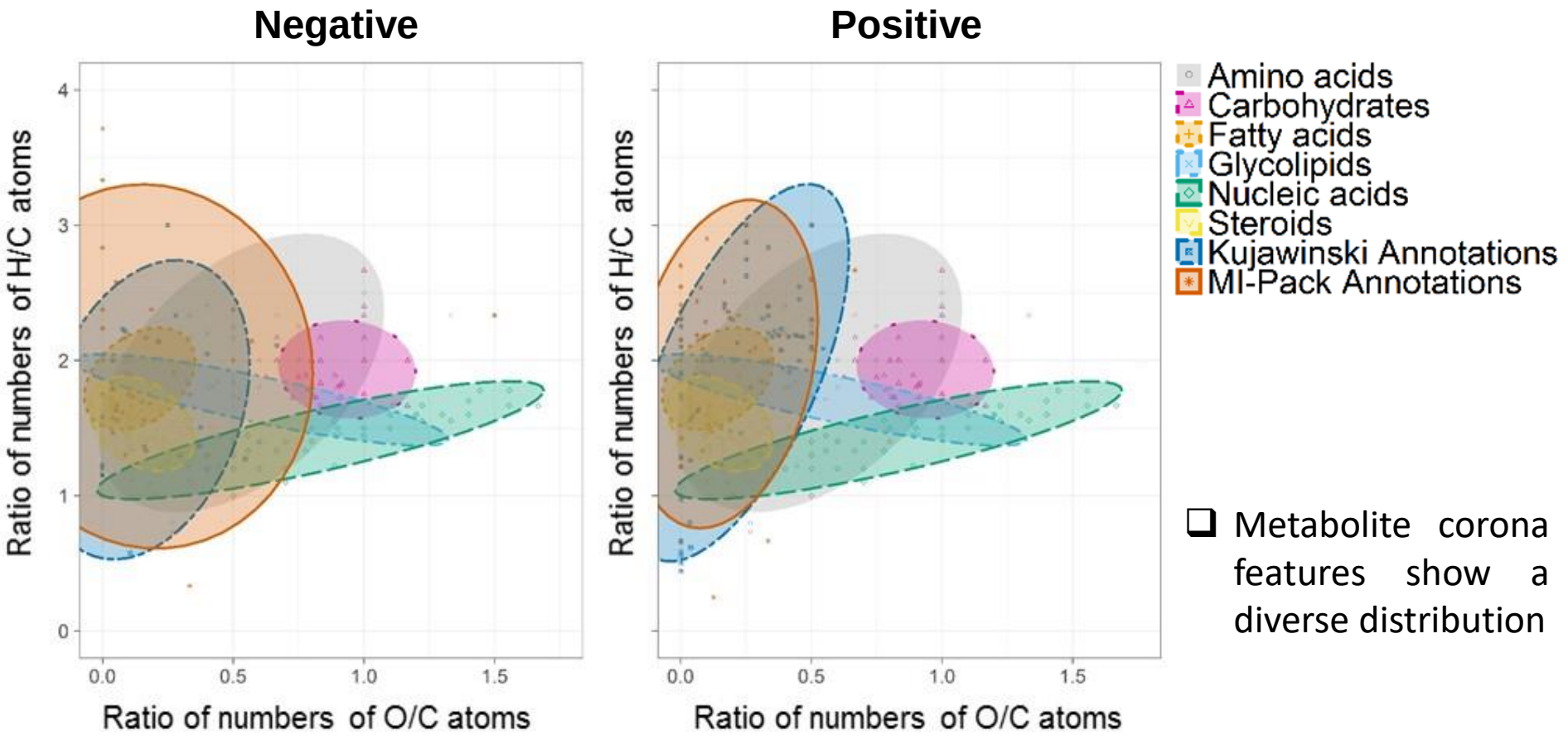
- Extraction in methanol:water is more efficient than methanol:formic acid.
- 2 extractions are sufficient to extract all signal of bound SDS.

Results and Discussion: Corona detection



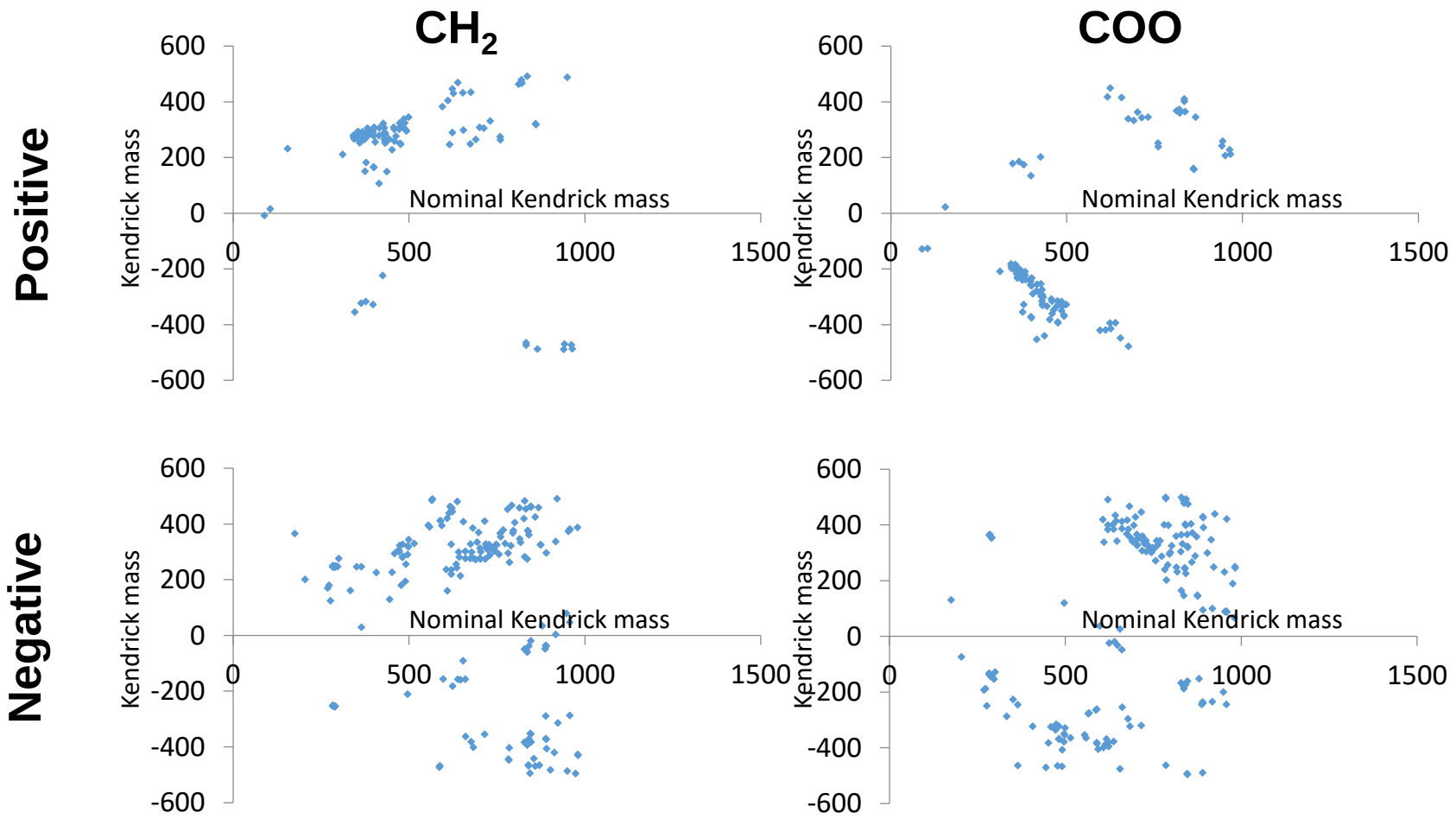
Wash steps
Extraction
Re-suspension
DIMS detection of metabolites

Results and Discussion: Corona detection

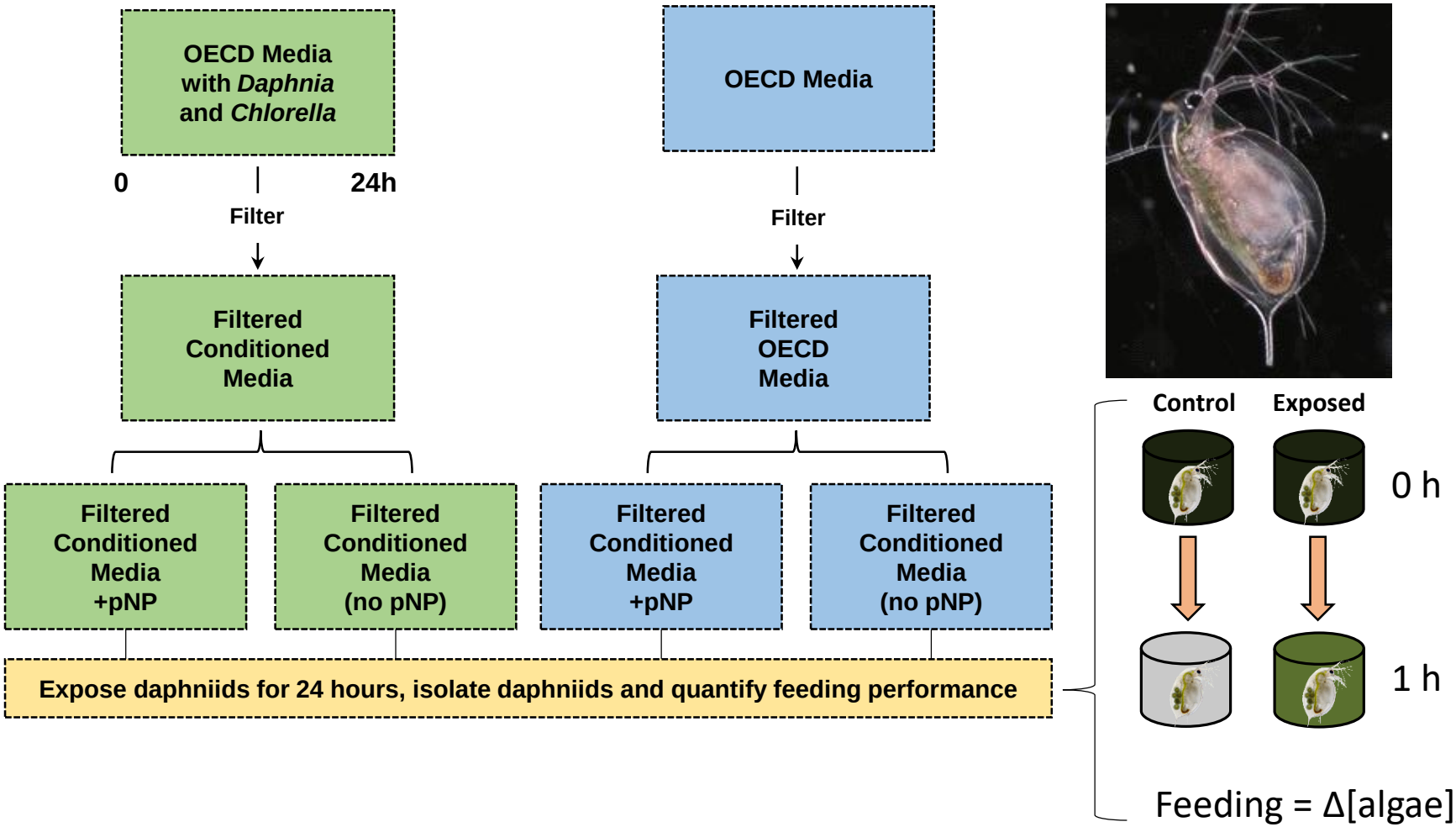


- Metabolite corona features show a diverse distribution

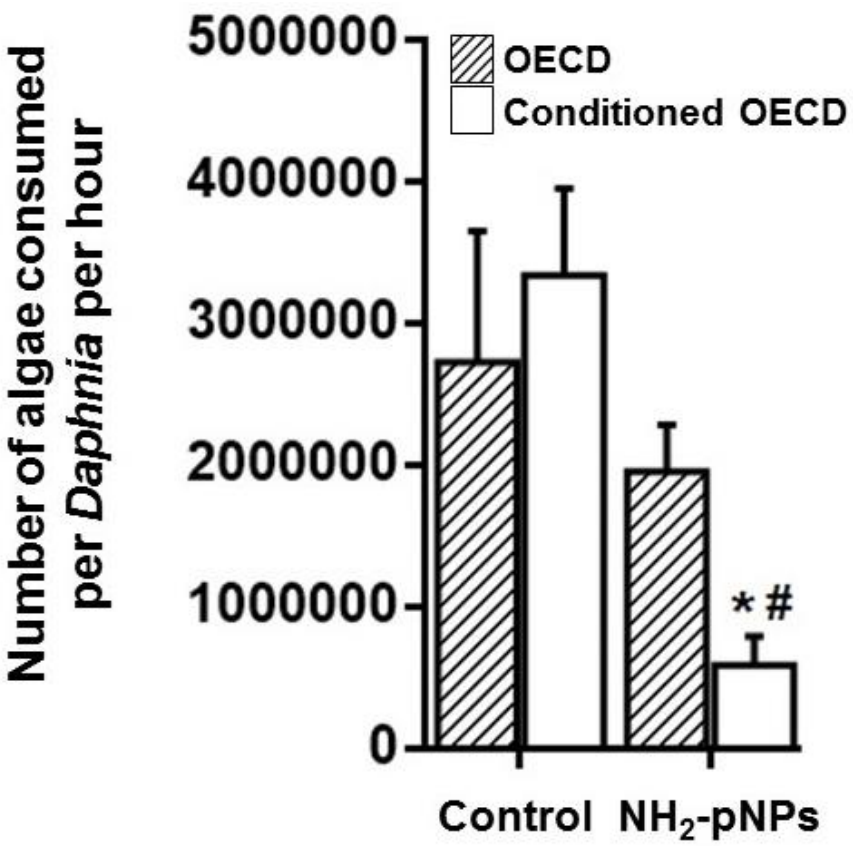
Results and Discussion: Corona detection



Results and Discussion: Corona impact on *Daphnia*



Results and Discussion: Corona impact on *Daphnia*



- Results show that exposure to NH_2-pNPs decreased the feeding performance in conditioned and unconditioned media by 82% and 29% (not significant by unpaired *t*-test compared to unexposed animals), respectively.
- It is evident that NH_2-pNPs incubated in conditioned media had a stronger impact on feeding performance and this could be attributed to the corona formed which makes NH_2-pNPs more accessible to daphniids as they filter their surrounding media during exposure.

Conclusions

- ✓ We present the development and application of an optimised protocol for the semi-quantitative detection of a small molecule metabolite corona from amino functionalised polystyrene nanoparticles.
- ✓ The new method reproducibly detected metabolites that were present within a freshwater model ecosystem (of *Daphnia* and algae) and that had subsequently bound to the NH_2-pNPs.
- ✓ This is the first attempt to demonstrate the existence of such an ecologically relevant corona on the surface of NPs and also revealed its importance in freshwater organisms using phenotypic endpoints (feeding).

Acknowledgements

FUTURE NANO NEEDS

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