SUPPORTING INFORMATION

Surface Immobilisation of Engineered Nanomaterials for in-situ Study of their Environmental Transformations and Fate

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Particle Size Distribution analysis by ImageJ

Particle sizes were analysed using the ImageJ software (ver. 1.46r) and its built-in Particle Analysis function on the SEM images acquired at ×300,000 magnification. Images were imported into ImageJ in TIFF format, despeckled to reduce the noise, and converted to a binary format by applying a manual threshold prior to Particle Analysis. Scales were set using the bar on each image. The results were opened in a spreadsheet, sorted in order of particle area, and data was truncated at low (due to pixilation and resolution limit) and high (aggregated particles) particle areas. Nominal particle sizes were determined by assuming a spherical geometry and calculating their diameters from their areas ($d_{nom} = 2 \times (A/\pi)^{\frac{14}{5}}$). The arithmetic mean of d_{nom} , in nanometres (nm), is reported as the number-weighted average size.

Figure S1. Scanning Electron Micrscopy (SEM) images (SE/BSE overlay) of the control substrates, particle size distributions (PSD) and Energy Dispersive X-ray (EDX) spectra:

i) CIT-AgNPs at a) ×80,000, and b) ×300,000 magnification, with c) the PSD as determined from
b), and d) EDX spectrum (10 keV, 100 sec acquisition).



ii) PEG-AgNPs at a) ×80,000, and b) ×300,000 magnification, with c) the PSD as determined from b), and d) EDX spectrum (10 kV, 100 sec acquisition)



iii) SE mode SEM images of PEI-AgNPs at a) ×80,000, and b) ×300,000 magnification, with c) the PSD as determined from b), and d) EDX spectrum (10 kV, 100 sec acquisition)



Figure S2. SEM images of all immobilised AgNP samples: a) control, b) primary SS, c) freshwater media (OECD), d) freshwater lake, e) saltwater media, and f) sea (marina), for:

i) CIT-AgNPs



1 µm

500 nm

500 nm





Figure S3. XANES spectra of Ag standards used in the linear combination fitting (LCF) analysis of CIT-AgNPs exposed to various conditions. The same set of standards were used for PEG- and PEI-AgNPs, but with the corresponding control AgNPs.

Ag bound to cystine (Ag-Cys) or glutathione (Ag-GSH) were the organically bound standards. Inorganic standards included silver sulfide (Ag₂S), silver chloride (AgCl), silver sulfate (Ag₂SO₄) silver carbonate (Ag₂CO₃) and silver oxide (Ag₂O), as well the control AgNPs.



Figure S4. Raw XANES profiles (pre-normalisation) of all samples showing the different absorbance magnitude after exposure, suggesting some loss of silver (as Ag⁺, AgNP, or otherwise) from the surface. Note that the pre-exposure variability in the AgNPs attachment density is *ca.* 10 %.



i) CIT-AgNPs





