Capturing the colloidal microplastics with plant-based nanocellulose networks

Author list

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Abstract

Microplastics accumulate to various aquatic organisms causing serious health issues, and they have raised concerns about human health by entering our food chain. The recovery techniques for the most challenging colloidal fraction even for the analytical purposes are limited. Here we show how hygroscopic nanocellulose network acts as an ideal capturing material even for the tiniest nanoplastic particles. We reveal that the entrapment of particles from the aqueous environment is a result of the network’s hygroscopic nature - a feature which is further intensified with the high surface area. We determine the nanoplastic binding mechanisms using surface sensitive methods, and interpret the results with the random sequential adsorption (RSA) model. The microplastic uptake does not rely on any specific interfacial interaction but rather on the water transport behavior of nanocellulose. These findings hold potential for the explicit quantification of the microplastics.
from different environments, and eventually, provide solutions to collect those directly on-site where they are produced.

Plastic pollution entering our environment at an increasing rate is a major problem, especially in the marine environment\(^1\). It is estimated that 8.8 million tons of plastic waste end up in oceans every year\(^4\). Primary micro- (\(\mu\)Pp, size 1\(\mu\)m - 5mm) and nanoplastic (nPp, size <1\(\mu\)m) particles used in i.e. pharmaceuticals and cosmetics can enter the environment directly. Erosion of plastics causes fragmentation into smaller particles, namely secondary micro- and nanoplastic particles\(^5,6\).

Nanoplastics are especially harmful due to their small size (hard to capture, can enter cells), large surface area (capable to bind e.g. toxins), and colloidal nature (limited means for quantification and qualification).\(^5\) A few studies have analysed their presence in aquatic animals such as fish and molluscs where they have been found and quantified proving their existence.\(^7\) A recent study has also shown their presence in human placenta\(^8\). Model nPp accumulate on algal cell surfaces\(^9\), to various organs in mussels\(^10\), and to juvenile zebra fish\(^11,12\) affecting their quality of life. An extensive amount of knowledge on the abundance of microplastic particles in different environments is available\(^13\). Recent efforts to overcome the plastic challenge highlight how genetically engineered enzymes can degrade plastics.\(^14\) This approach could also be a tool for microplastic management. However, very little is known about the existence of nanoplastic particles mainly due to the technical challenges associated with their capture, separation, and analysis\(^15\) – \(^17\).

To date, there is no means to recover nPp from environmental samples for explicit quantification or for the qualitative analysis since existing methods are based on different filtration and elutriation techniques appropriate only for the larger-sized \(\mu\)Pp (>50\(\mu\)m)\(^6,18,19\). At best, particle diameters varying from few microns up to tens of microns can be extracted via density flotation and methods which are based on migration velocity differences\(^20\). These restrictions leave a blind spot for the recovery, quantification, and qualification of colloidal plastic particles (\(\phi < 1\mu m\))\(^16\). Here we show that lignocellulose-based networks can be harnessed to qualify and quantify even the most
challenging fraction of the colloidal plastics. Plant-sourced cellulose nanofibrils (CNF) are colloidal level objects with lateral dimensions of 3-10 nm and length up to micrometers. Their water-responsive nature, self-assembly, and other unique properties have only recently unraveled.\textsuperscript{21} More specifically, they can effectively recover e.g. gold ions from waste waters\textsuperscript{22} and interact with nanoparticles in general\textsuperscript{21}. In the realm of nanoscaled materials, besides hydrophilicity and abundance in nature, the assemblies are highly hygroscopic. Strong interactions with water distinguish nanocelluloses from many other nanomaterials with similar properties in terms of large surface area and high aspect ratio.\textsuperscript{23}

We evidence the capturing ability of nanocellulose networks by following the fluorescent intensity of microplastic particles either in microfluidistic set-up or by simply using nanocellulose films as elements to collect the particles from the aqueous dispersions. We utilized model polystyrene (PS) particles with different surface charge and size, i.e. anionic and cationic µPp (\(\phi = 1.0/1.1\mu m\)) and anionic and cationic nPp (\(\phi = 100\text{nm}\)) (Supplementary Table 1) to reveal the essential mechanisms facilitating the capturing efficiency. We introduce an interfacial approach where particle adsorption data is coupled with the sequential adsorption model, and hence, we are able to quantify the particle uptake with kinetic information and provide a methodology for nanoparticle detection. Therefore, with our approach, we tackle the critical challenges related to reliable microplastic quantification, and we put forward the solutions for microplastic management where e.g. nanocellulose-based filter systems are capable to capture plastic particles on-site before entering the environment.

**Nanocellulose hydrogels trap microplastic particles**

We followed the microplastics capturing capacity of native CNF hydrogels\textsuperscript{24} in real-time using microfluidistic analysis coupled with fluorescent imaging (Fig. 1a-b), a straightforward and semi-quantitative concept to evidence the ability of nanocellulose hydrogel to trap polystyrene (PS) nano- and microplastic particles (cationic/anionic nPp and µPp). The fluorescence intensity increased in the microfluidic traps containing CNF with each cycle of particles of both size classes (Fig. 1c, d,
Supplementary Fig. 1, Extended Data Video 1 for positively charged particles, Supplementary Fig. 2 for negatively charged particles. Accumulation of the fluorescence intensity over time was as much as 70% higher for the positively charged nPp system when compared to µPp system (Fig. 1c, d). Also, the same applied to the negatively charged particles, however, the overall fluorescence accumulation of negatively charged particles (Supplementary Fig. 2 and 3) was lower compared to positively charged particles (Fig. 1c, d, Supplementary Fig. 3) and the difference between negatively charged nPp and µPp accumulation was not as large as with the positively charged particles. By analyzing the profile of the accumulated fluorescence within the CNF hydrogel (Supplementary Fig. 3a) we saw that the nPp were able to penetrate the hydrogel deeper compared to µPp, indicating that the hydrogel network is porous at the nanometer to micrometer scale (Supplementary Fig. 3b). The charges seems not to play a great role in penetration into the hydrogel network but seems to affect the quantity of captured particles (Supplementary Fig. 3b). Generally, the fluorescence intensity of single microplastic particle is significantly higher than that of nanoplastic particle, and thus, our results indicate that CNF hydrogel has a considerably higher capability to capture nPp than µPp.

Hygroscopic nanocellulosic assemblies display peculiar water transport properties involving capillary action and diffusion\(^{23}\). With the aid of water flux, the smallest microplastics seem to be conveyed inside the CNF hydrogel network. Moreover, the large surface area of the porous network enhances cohesion facilitating the entrapment of microplastics\(^{25}\). The negative overall charge of CNF also promotes the accumulation of positively charged particles, however, it does not prevent the accumulation of negatively charged particles.

**Capturing of micro- and nanoplastics with nanocellulose films**

We assessed the ability of nanocellulose films to collect nPp and µPp using fluorescence spectroscopy. This method allows the direct quantification of the number of microplastics from the aqueous dispersion before and after immersion of the films (Supplementary Fig. 4). By using...
particles with either anionic or cationic charge, we are able to further elucidate the capturing
mechanisms, i.e. whether the electrostatic interactions play a role along with the nanocellulose
network hygroscopicity. We used native CNF\textsuperscript{24} and TEMPO-oxidized CNF\textsuperscript{26} (Supplementary Fig.
5) - the grades with altered water responding tendency due to the phenomenon called
hornification\textsuperscript{27}. Due to the low anionic charge of native CNF (0.04 mmol g\textsuperscript{-1}) it tends to lose its
active surface area upon drying whereas the charge of TEMPO-CN F is remarkably higher (1.3
mmol g\textsuperscript{-1}) preventing the hornification and, therefore, the water responsive nature is retained.

The number of captured nPp and \(\mu\)Pp per unit area of nanocellulose films is shown in Fig. 2a and b,
respectively (Supplementary Table 2). SEM images in Fig. 2c show the appearance of the films
after being in contact with nPp dispersion. The polymeric regenerated cellulose (RC) and
hydrophobic polystyrene (PS) films were used as reference materials (Supplementary Fig. 5). These
results deliver two main messages: (i) highly hygroscopic and anionic TEMPO-CN F film performs
the best (Fig. 2a, b) in all cases. Surprisingly, the anionic nPp are most efficiently removed by the
TEMPO-CN F film. (ii) Attractive electrostatic interactions seem to have a more pronounced effect
when dealing with \(\mu\)Pp as anionic cellulose films capture cationic \(\mu\)Pp in larger quantities compared
to anionic \(\mu\)Pp (Fig. 2b).

As expected, due to the larger attractive energy between oppositely charged surfaces, the positively
charged particles are immediately attached to the anionic TEMPO CNF surface hindering the
particle diffusions inside the network, although not fully preventing it. In the anionic system, the
attractive energy between negatively charged surfaces is half of that of the oppositely charged
surfaces\textsuperscript{28}, and therefore, anionic nPp can enter inside the nanocellulose network more efficiently.
Indeed, TEMPO-CN F films capture approximately a third more of anionic nPp than cationic nPp.
The fact that TEMPO-CN F is able to efficiently capture nPp despite the particle charge is a
consequence of its nanoscaled porosity coupled with high hygroscopicity enabling peculiar water
transport properties. Once the film is in contact with aqueous solutions it swells drastically.
TEMPO-CNf network swelling induces capillary flow which is strong enough to transport nPp into the film network. The large and active surface area of native CNF is partly lost during drying since this CNF grade has a significantly lower surface charge when compared to the TEMPO-CNf. Due to the lack of repulsion between the individual fibrils, upon drying, fibrils aggregate, and severe hornification takes place. Hornification causes irreversible changes in fibril morphology and specific surface area reducing the swelling of the fibril network and thereby lowering the water uptake ability of the system.\(^{27}\) Thus, hornification explains the lower performance of native CNF films compared to TEMPO-CNf films since never-dried CNF hydrogels are able to recover microplastics from water flux as discussed above (see Fig. 1). A significantly smaller area of the TEMPO-CNf film (30 cm\(^2\)) is needed to remove all anionic nPp from the solution when compared to the native CNF film (140 cm\(^2\)) (Supplementary Table 3).

Our results show that all films recover more of the cationic µPp than the anionic ones (Fig. 2b, Supplementary Table 2) indicating a more pronounced role of attractive electrostatic interaction in the capture process compared to the nPp system. Nanocellulose-based systems, however, outperform the polymeric systems based on regenerated cellulose and polystyrene (RC and PS), due to large surface area, high hygroscopicity, and possible entrapment of particles in the porous network. Lignocellulose-based systems indeed can efficiently trap and transport microplastics from seawater (~1500 plastic pieces/kg of seagrass) as demonstrated in the seagrass ecosystem\(^{29}\). At best, our system - also lignocellulose-derived - can collect roughly 20B nPp/mg TEMPO-CNf, which is a remarkable finding. The ability of regenerated cellulose film to sieve particles cannot be fully explained either by attractive electrostatic interactions, large surface area, nor water interactions and, therefore, we assess the role of direct surface interactions in the capturing process.

**The role of interfacial interactions - Quantitative method to calculate the adsorption parameters**
To further elaborate the role of surface interactions between nanoplastics and the binding substrates, the adsorption of anionic nPp particles was followed using Quartz crystal microbalance with dissipation monitoring (QCM-D). With this approach, we aim to exclude the influence of network porosity that generates water transport functions and amplify the role of direct surface interactions. We focus our interfacial investigations on colloidal-sized nPp particles since the behaviour of nanoscaled particles is mostly taking place at interfaces. In nature, nanoplastics tend to accumulate e.g. toxins and therefore from the environmental point of view, pure particles do not exist. Therefore, we used either stabilised or purified PS particles, all carrying a net negative surface charge (Supplementary Table 1).

Our results show that anionic nPp particles - both stabilised and purified - adsorb on native CNF, regenerated cellulose (RC), and on polystyrene (PS) ($\Delta f_{RC} >> \Delta f_{CNF} > \Delta f_{PS}$) (Fig. 3a and Supplementary Fig. 6 a,c) whereas no adsorption was detected on TEMPO-CN. This result indicates that the electrostatic repulsion between anionic domains prevents the direct binding of nPp on the highly anionic TEMPO-CN.

Finally, we introduce a systematic approach for explicit nanoplastic particle detection to bridge the well-known methodological gap of detection and quantification of nPp from the environment. We qualified the substrate performance to bind colloidal plastics via surface interactions by comparing the experimental surface coverage to the theoretical maximum coverage. This was carried out by linking the adsorption data to comprehensive image analysis and by applying a random sequential adsorption (RSA) model. In the RSA model, the jamming limit at which the density of adsorbed particles (particles treated as geometrically restricted and fixed circular objects without conformational and orientational changes) saturates on a 2D film is defined as a theoretical maximum coverage ($\theta_\infty = 0.547$). Therefore, the saturation limit in RSA is significantly lower than the optimum filling of the surface. By fitting the QCM-D data (Fig. 3a) with the RSA model (Fig. 3b), and by applying the image analysis (Fig. 3c, Supplementary Fig. 7-9) we gain access to
the adsorption rate and the number of particles per unit area after the adsorption \((dN/dt)\) (Supplementary Table 4, Equation 2), which can be translated to surface mass density \((\Gamma)\) and adsorption rate \((d\Gamma/dt)\) (Equation 6) since the nanoplastic adsorption process meets well the RSA requirements (See Methods). QCM-D detects the adsorbed hydrated total mass by acoustic principle showing simultaneously high changes in energy dissipation (Supplementary Fig. 6 b,d and 10).

Therefore, the simple Sauerbrey equation (Equation 1) cannot be directly used to calculate the mass of the adsorbed particles. To quantify the amount of water in the adsorption process, the areal mass generated from QCM-D data were rescaled utilizing the surface mass density \(\Gamma\) determined from the SEM images by image analysis to quantify the dry mass of adsorbed nPp. Fig. 3c illustrates the appearance of different substrates after the nPp adsorption process and displays the recognition of particles in order to analyse the experimental coverage \((\theta_{\text{max}})\) and maximum surface mass density \((\Gamma_{\text{max}})\) via image analysis. Table 1 collects the relevant experimental data on particle adsorption, a factor describing water coupled to the adsorbed layer, and surface coverage \((\theta_{\text{max}})\) at the solid-gas interface at the end of the irreversible adsorption process \((t \approx 1h)\). Table 1 also tabulates the system-specific parameters from RSA fittings i.e. surface coverage \((\theta_{\text{max}})\) at the solid-liquid interface, the adsorption rate coefficient \((k_a)\), and the occupied area \((a)\) of single nPp including the water, which is strongly interacting with the nanoplastic particle. It should be noted that RSA-derived \(\theta_{\text{max}}\) takes into account the particle diameter with coupled water resulting in higher surface coverage values when compared to “dry systems”. Our results show that the strongly bound water layer does not prevent the particle packing and therefore the estimation of the true surface coverage \((\theta_{\text{max}}/\theta_{\infty})\) using dry system data is warranted. The full treatise of the adsorption data with the RSA model is described in the Methods and Supplementary Fig. 11.

We extracted the key findings, and the discussion is supported by the schematic presentation shown in Fig 3e. (i) Nanoplastics have the highest affinity and the highest probability (high \(k_a\)) to attach on regenerated cellulose suggesting favorable surface interactions positively contributing to adsorption.
Particles on regenerated cellulose seem to occupy a smaller area (a) allowing a closer packing density indicating that only the synergetic effect of the hydration shell and the electrical double layer of the particles limit the particle packing. Approximately 50% of the theoretical surface coverage maximum can be achieved within the time scale of one hour (Fig. 3d). (ii) Low anionic charge of native CNF substrate promotes nPp adsorption and direct binding of particles. Approximately 15% of the theoretical surface coverage maximum can be achieved with CNF, and the nature of the particle - whether purified or stabilised - has only a minor influence on capturing behaviour. (iii) The chemical compatibility and the hydrophobic nature of polystyrene seem not to increase the probability of nanoplastics to adsorb on polystyrene. Nanoplastics have the lowest adsorption probability (low $k_a$) on polystyrene although the probability significantly increases in the purified nPp system. A circumspect explanation for the high area (a) occupied per particle at the solid-liquid interface is originating from the higher amount of coupled water per adsorbed particle indicated by the factor of water being 4 for polystyrene systems (Table 1). The amount of coupled water (the water factor of 3) corresponds to approximately 2/3 of the experimentally sensed total mass for CNF and RC surfaces and 3/4 (the water factor of 4) for the PS surface. This is in accordance with other methods. Simultaneously, the changes in dissipation for PS systems reach relatively low values suggesting the dominating role of the bulk water over the coupled water in the system.

The adsorption behavior of nPp seems to be linked to the amphiphilic nature of cellulose, especially when the electrostatic repulsion of the system is relatively weak (native CNF and regenerated cellulose). Cellulosic materials have been shown to display different surface properties due to the structural anisotropy. Receding and advancing contact angle values are ranging between 11° and 37° suggesting that that cellulose has both hydrophobic and hydrophilic domains. Different wetting behavior of the cellulose surfaces has been shown to correlate with the orientation of the crystal planes. The mechanism defining the nPp binding is based on the attractive surface
interactions only when the strong repulsive forces are not dominating and the capillary forces are not assisting the capturing process.

**Summary**

We introduce a universal and versatile nanocellulose-based solution, which efficiency to collect and bind micro- and nanoplatic particles is not dependent on any specific physical or chemical interaction. Instead, the entrapment of particles from aqueous dispersion is a result of a dual synergistic feature provided by the nanocellulose network: high hygroscopicity coupled with a high active surface area. These attributes enable the operational assets where colloidal plastic particles - regardless of the size or surface chemistry - can be conveyed and captured inside the cellulosic network by exploiting its peculiar water transport properties involving capillary forces and diffusion. Once inside the network, the large surface area and favourable surface interactions enhance cohesion between the particles and the surface of the material leading to efficient capturing. We show that by combining surface-sensitive methods with nanomicroscopy, image analysis, and modeling, we are able to quantitatively assess the nPp behavior at interfaces. This type of nPp adsorption data has not been previously collected, and it is essential when designing materials for quantitative analysis purposes, and for collection and recovery from different environments ranging from wastewaters to the sites where the nano- and microplastics are produced. Nanocellulose originates from the natural sources, it is renewable and non-toxic, which are key aspects when designing next-generation functional materials diminishing the dependency on the synthetic counterparts. Today, nanocellulose can be produced and modified in various ways to yield hydrogels, self-standing films, and porous aerogels and cryogels, which make it an ideal material for many future solutions where the high hygroscopicity is an asset.

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Figure 1. Capture of microplastic particles by native CNF hydrogel network. a, Schematic illustration of a proof of concept where the capture of fluorescently labelled microplastic particles by CNF hydrogel network is verified using a microfluidic set-up and fluorescent imaging (Extended Data Video 1). b, Schematic illustrations of the microfluidistic setup for CNF containing trap showing the injection of fluorescent nPp and µPp (I-3) and water (I-2/I-1). I-1 channel is used to pack the CNF hydrogel into the connected traps and I-2 is used for washing. Fluorescent accumulation of nPp (c) and µPp (d) over time by CNF hydrogel network. Green curves show control trap without CNF hydrogel. The orange and blue curves show parallel experiments with CNF in the traps. In d, the red dots indicate the time points where microscopy images were taken (Supplementary Fig. 1).
Figure 2. Quantitative assessment of entrapped fluorescent microplastic particles of different size and charge by self-standing films. a, Number of captured nPp and b, µPp calculated based on the fluorescence detection. White bars represent negatively charged plastic particles, and grey bars positively charged plastic particles. The full data for all captured particles is presented in Supplementary Table 2. c, SEM images of the films after being contacted with the aqueous nPp dispersion for 10 min. Scale bar in SEM images is 1µm.
Figure 3. Quantitative assessment of surface binding of nPp using surface sensitive approach coupled with image analysis and fittings with random sequential adsorption (RSA) model. a, QCM-D frequency change responses showing the adsorption of stabile nPp on TEMPO-CNf (dark gray line), PS (grey line), native CNf (solid black line), and RC (dashed black line). b, Fitting of the QCM-D adsorption data of stabile nPp on CNf using the RSA model. Black dots are measured data, and black line is the RSA fit. The adjusted R² for the fit is 0.91. c, Stabile nPp recognition from scanning electron microscopy (SEM) images using image analysis (Supplementary Fig 9). The SEM image scale bar is 0.5 µm. d, Amount of nPp detected after the adsorption experiments (white bars stabile, grey bars purified) via image analysis of SEM micrographs contrasted to a theoretical surface coverage maximum (θ∞ = 0.547 which equals to ~5.8 × 10⁻⁷ circles mm²), which is based on the RSA model. e, Schematic presentations display the appearance of substrates at the end of the nPp adsorption process showing the existence of bulk water and water which is strongly interacting with nPp. d_eff describes the effective particle diameter, and d_RSA is the diameter of the occupied area (a) of a single nPp including the particle and the coupled water.
### Table 1

Experimental data, adsorption parameters and surface coverage estimations for different nPp systems.

|                  | Experimental data | RSA fitting parameters |
|------------------|-------------------|------------------------|
|                  | QCM $\Delta f_5$ (Hz)$^a$ | QCM $\Delta D_5$ (×10$^6$)$^a$ | Coupled water,$^b$ $\Delta f_5(QCM)$ $\Delta f_5(IA)$ | $\Gamma_{max}$ (ng cm$^{-2}$) | $\theta_{max}$ (solid/air)$^d$ | $\theta_{max}$ (solid/liquid)$^e$ | $a \times 10^4$ (nm$^2$)$^f$ | $d_{RSA}$ (nm)$^g$ | $k_a \times 10^{-5}$ (cm s$^{-1}$)$^h$ | $\theta_{max} / \theta_\infty$ |
| CNF + ⚪         | -75               | 21                     | 3.2                     | 419           | 0.076       | 0.48   | 6.0   | 280   | 1.0   | 0.14  |
| CNF + ⚫         | -92               | 24                     | 3.1                     | 524           | 0.095       | 0.45   | 4.5   | 240   | 1.2   | 0.17  |
| RC + ⚪          | -230              | 38                     | 3.1                     | 1330          | 0.24        | 0.35   | 1.4   | 130   | 1.6   | 0.44  |
| RC + ⚫          | -340              | 47                     | 3.2                     | 1890          | 0.34        | 0.34   | 1.0   | 110   | 1.9   | 0.62  |
| PS + ⚪          | -19               | 5.4                    | 4.2                     | 80.7          | 0.015       | 0.36   | 24    | 550   | 0.2   | 0.027 |
| PS + ⚫          | -67               | 14                     | 4.2                     | 281           | 0.051       | 0.36   | 7.6   | 310   | 1.2   | 0.093 |

$^a$ Changes in frequency and dissipation at the end of the QCM-D measurement after the rinsing step.

$^b$ Estimation of the amount of water detected at the end of nPp adsorption (see Methods).

$^c$ Maximum surface mass density gained from SEM images and image analysis.

$^d$ Maximum experimental surface coverage at the end of the adsorption experiment at solid/gas interface determined by image analysis. Particle amount on the surface compared to the theoretical maximum amount calculated based on the RSA model assuming that $\theta_\infty = 0.547$.

$^e$ Maximum experimental surface coverage at the end of adsorption at solid/liquid interface calculated using Equation 4, where area $a$ is obtained from RSA fitting.

$^f$ Occupied area of a single nanoplastic particle including particle and the coupled water i.e. water strongly interacting with the particle

$^g$ Diameter of the area (a) taken by the particle and coupled water

$^h$ Adsorption coefficient describing the affinity of nPp towards the surface obtained from RSA fitting.

$^i$ Fractional surface coverage, where $\theta_\infty = 0.547$ is the theoretical maximum surface coverage based on the RSA model.

When analyzing the adsorption of purified nPp on RC substrate, Equation 3 is valid when $\theta_{max} < 0.3$. Since $\theta_{max}$ for RC is > 0.3, the Equation 5 was applied. If applying the Equation 6, $d_{RSA}$ would be 96 nm, which is an underestimate since $d_{eff} = 110$ nm.
METHODS

Polystyrene particles

Fluorescently labeled polystyrene (PS) particles (L9902, L9904, L4655, and L9654 from Sigma Aldrich) of different size and surface charge (cationic and anionic) were used to analyze microplastic capturing ability, and to reveal the capturing mechanisms of CNF hydrogel and self-standing films. Particles with diameters of 100 nm and 1.0 µm (i.e. nanoplastic particles nPp and microplastic particles µPp, respectively) were used. Later referred to as fluorescently labeled nPp or µPp or fluorescently labeled particles if both sizes are discussed.

Colloidal sized PS particles (LB1 from Sigma Aldrich) were utilized for QCM-D experiments (ø = 100 nm, i.e. nPp). These particles were utilized in a stable form (i.e. utilized as provided by the supplier) or purified with the supplier's protocol to remove most of the stabilising agent. Stable particles represent the native state of nanoplastics. Surface charges for the stable and purified particles were determined by zeta potential measurements (Supplementary Table 1).

Nanocellulose materials

Two different grades of cellulose nanofibrils were utilized: mechanically disintegrated cellulose nanofibrils (native CNF)\textsuperscript{24} and TEMPO-oxidized (TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy radical) cellulose nanofibrils (TEMPO-CNФ).\textsuperscript{26} These two nanocellulose materials vary in fibril diameter, surface roughness, and water contact angle (Supplementary Fig. 5). Mechanically disintegrated CNFs were prepared from never dried bleached birch kraft pulp obtained from the Finnish pulp mill. The pulp was first soaked at 1.7 wt% consistency and dispersed using a high shear Diaf dissolver (Minibatch Type20) for 10 min at 700 rpm. The pulp suspension was pre-refined in a Masuko grinder (Supermasscolloider MKZA10-15J, Masuko Sangyo Co., Japan) at
1500 rpm and fluidized with six passes through a Microfluidizer (Microfluidics M-7115-30 Microfluidics Corp.). The mechanical disintegration resulted in a viscous gel with a final solid content of ~1.6 wt% with an anionic charge of 0.04 mmol g\(^{-1}\) analysed by conductometric titration (SCAN 65:02).

TEMPO-oxidized cellulose nanofibrils (TEMPO-CNf) were produced from bleached softwood kraft pulp obtained from the Finnish pulp mill. Prior to the fibrillation, the pulp was TEMPO-oxidized according to a protocol described by Saito et al.\(^{26}\) Shortly, the never-dried bleached softwood pulp was oxidized with NaClO mediated by 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, Sigma Aldrich). The degree of oxidation per anhydroglucose unit was 1.3 mmol g\(^{-1}\), determined by conductometric titration (SCAN 65:02). The washed TEMPO-oxidized pulp was subsequently fibrillated with a high-pressure fluidizer (Microfluidics M110P, Microfluidics Int. Co., Newton, MA) with two passes. The final solid content was 1 wt%.

**Microfluidistic set-up and fluorescence microscopy**

The capacity of native CNF hydrogel to capture microplastic particles (fluorescently labeled) was followed using a microfluidic setup and fluorescence microscopy. Microfluidic chips were designed and prepared by standard soft lithography and replica molding approach as previously described\(^{38}\). Initially, a master mold including photoresist SU-8 (MicroResist GmbH) on a silicon wafer was created by spin coating two distinct layers, SU-8-5 for the 1.6 \(\mu\)m layer and SU-8-50 for the 20 \(\mu\)m layer. Each layer was exposed to a mercury lamp i-line (3 & 8.5 seconds, respectively). After development of the topographies, the surface was coated with a ~20 nm fluorocarbon polymer film to facilitate the removal of the PDMS replica after molding\(^{39}\). PDMS was prepared by mixing the monomer and crosslinking agent in 10:1 ratio (Sylgard 184 kit, Dow Corning), degassing it, casting it on the microfluidic mold, followed by an overnight curing step at 70°C. Chips were bonded to glass coverslips by oxygen plasma treatment.
CNF dispersion of 0.5 wt% was prepared for the microfluidic experiments. Before loading the microfluidic channels, the 0.5 wt% CNF solution was centrifuged for 5 min to spin down large fibrils. The supernatant was loaded into the microfluidic chips with a 500 µl syringe through a single channel, with a flow rate of 300 µm s\(^{-1}\). After the CNF fibrils entangled behind the pillars to form a solid transparent membrane, the CNF solution was switched to H\(_2\)O. Phase-contrast and fluorescent images were acquired using an Axio Observer Z1 microscope (Carl Zeiss, Jena, Germany)\(^{40}\). Images were acquired every two minutes at 20x magnification during the experiment. The fluorescent signal was obtained from the fluorescent 100 nm and 1.0 µm particles using excitation light at 480 nm, while collecting the emitted light from 515-535 nm. The flow was continually switching between the wash solution (Channel B) and the microplastic particle solution (Channel C) every six minutes.

A cross-section profile fluorescence intensity analysis of the CNF hydrogel network was performed on 4 individual traps for each condition in order to gain understanding of the penetration of nPp and µPp into the CNF hydrogel network. Cross-sections were acquired in the middle of the washing cycle at 86 min, to ensure only entrapped and bound particles were considered in the analysis. Fluorescence reads were scaled against its peak fluorescence, after which the area under the curve was calculated (Δx 400 nm) and plotted against the distance of the trapped nPp and µPp.

**Preparation of self-standing films**

Native CNF films were prepared from 0.8 wt% gel, which was cast on polystyrene Petri dishes (φ 9 cm) and dried under ambient conditions for 24 hours. The formed CNF films were separated from the plastic supports for further experiments.

TEMPO-CNFFilms were prepared from 0.2 wt% gel, which were cross-linked with polyvinyl alcohol (PVA, Mowiol 56-98, M\(_w\) 195 000g/mol) according to previously described procedure\(^{41}\) to enhance the films’ wet strength. TEMPO-CNFF gel was cast on polystyrene Petri dishes (φ 9 cm)
and dried under ambient conditions for 24 hours. The formed TEMPO-CNФ films were separated from the plastic supports for further experiments. The weight of 1.5 cm x 1.5 cm film was on average 0.0047 g.

RC film was prepared by dissolving microcrystalline cellulose powder from spruce (Fluka) in ionic liquid (EMIM[OAc], IoLiTec GmbH) to a 10 wt% solution under heat and mixing. Subsequently, a film was cast on a glass surface using a 510 Coatmaster film applicator (ERICHSEN GmbH & Co. KG, Hemer, Germany) with a gap of 400 μm and speed of 40 mm s^{-1}. The regeneration was carried out by immersing the cellulose in water for 1.5 hours. Finally, the regenerated cellulose film was placed between absorbent papers and air-dried at ambient conditions for 3 days.

PS film was prepared by dissolving PS pellets (Mw 192,000 g/mol, Sigma Aldrich) in toluene to a 10 wt% solution at ambient conditions overnight. The PS solution was cast on a glass Petri dish and air-dried at ambient conditions for 12 hours. The formed PS film was separated from the glass support for further experiments.

**Fluorescence spectroscopy**

Quantification of fluorescently labeled anionic and cationic particles captured by self-standing films was conducted using a Cary Eclipse fluorescence spectrophotometer (Varian Scientific Instruments, CA, USA). Fluorescently labeled particles were dispersed to a final concentration of 0.1 g L^{-1} in phosphate buffer (10 mM, pH 6.8). The calibration curves were recorded from five concentrations (0, 0.025, 0.05, 0.075, and 0.1 g L^{-1}) for each fluorescent particle at their specific emission maxima (Supplementary Fig. 12). Fluorescence studies were carried out by immersing the films in the aqueous dispersion containing 0.1 g L^{-1} particles for 10 min without mixing (Supplementary Fig. 4). The fluorescence of the solutions was recorded before and after the immersion. The amount of recovered particles was calculated by subtracting the fluorescence value after immersion from the
fluorescence before the film immersion. All measurements were performed in triplicate with three readings each.

The number of particles captured by the films equals to the total mass recovered \( (m_{\text{Tot}}) \) divided by the single-particle mass \( (m_{\text{particle}}) \). Above \( m_{\text{Tot}} = cV \), where \( c \) is the measured concentration, and \( V \) is the known volume. The mass of a single PS particle \( (m_{\text{particle}} = 5.49 \times 10^{-7} \) ng) was calculated, assuming it to be a sphere with a density of \( \rho = 1.05 \) g cm\(^{-3}\) \( (\rho \) of PS particles is provided by the supplier).

Preparation of ultrathin films for adsorption investigations

Native CNF, TEMPO-CNFS, RC, and PS ultrathin films were prepared by spin coating (Model WS-400BZ-6NPP/LITE, Laurell Technologies, North Wales, PA, USA) the materials on QCM-D sensor crystals (AT-cut quartz crystals with Au or SiO\(_2\) surfaces supplied by Q-Sense, Gothenburg, Sweden). The crystals were rinsed with Milli-Q water, dried with nitrogen gas and placed in a UV-ozonizer (Bioforce Nanosciences, CA) for 10 min to clean the surfaces. Prior to CNF or TEMPO-CNFS deposition, a layer of anchoring polymer (polyethylene imine (PEI), 30 wt%, Mw 50,000-100,000 g/mol, Polysciences Inc.) was adsorbed onto the crystal surface by immersing the crystal in 1 g L\(^{-1}\) PEI solution for 30 min. The excess of PEI was rinsed away with large amounts of Milli-Q water, followed by nitrogen gas drying.

Transparent dispersion of CNF for spin coating was prepared as described by Ahola et al.\(^{42}\) Briefly, CNF gel was ultrasonicated (400 W tip sonicator, Branson 450 Digital Sonifier, Branson Ultrasonics, Danbury, USA) for 10 min with 25 % amplitude. Subsequently, larger fibril bundles were removed by centrifugation (10400 rpm, 45 min). The supernatant with the individual fibrils was collected by pipetting. The Au-coated sensor surface with PEI was first wetted by spin coating 100 \( \mu \)L of Milli-Q water on the sensor at 3000 rpm for 10 s. Subsequently, the individualized nanofibrils were spin coated (3000 rpm, 90 s) on the QCM-D sensor surfaces. After spin coating,
the surfaces were rinsed with water, dried gently with nitrogen gas and placed in an oven for heat-
treatment (80 °C, 10 min).

Ultrathin films of TEMPO-CNf were prepared with a protocol described by Hakalahti et al.23. TEMPO-CNf surfaces were prepared from 0.15 wt% TEMPO-oxidized CNF solution, which was
ultrasonicated for 2 min with 25 % amplitude to break down aggregates. Before spin coating of
TEMPO-CNf the Au-coated sensor surface with a thin PEI layer was wetted by spin coating 100 µl
of Milli-Q water on the sensor at 3000 rpm for 10 s. Subsequently, the nanofibrils were spin coated
(3000 rpm, 90 s) onto the QCM-D crystals. After spin coating, the surfaces were rinsed with water,
dried gently with nitrogen gas, and placed in an oven for heat-treatment (80 °C, 10 min).

RC surfaces were prepared from trimethylsilyl cellulose (TMSC) ultrathin films by desilylation.
TMSC was synthesized by silylation of cellulose powder with hexamethyl disilazane (HDMS), as
described previously.43 Dried TMSC was dissolved into toluene to form a 10 g L⁻¹ solution. Prior to
spin coating the TMSC, the surface of the SiO₂-coated sensor surface was wetted by applying 5
droplets of toluene and spun with the speed of 3000 rpm for 15 s. The TMSC solution was
subsequently spin coated onto SiO₂-sensor (3000 rpm, 60 s). The solvent was evaporated by placing
the crystals in the oven (60 °C, 10 min). The TMSC ultrathin films were regenerated back to
cellulose by desilylation with hydrochloric acid vapor, producing ultrathin RC films.

PS ultrathin films were prepared from a polystyrene solution (0.1 wt% polystyrene in toluene) by
spin coating on a gold QCM-D crystal (4000 rpm, 30 s). The solvent was evaporated in the oven (60
°C, 10 min). All coated QCM-D crystals were stored in desiccators.

**Atomic force microscopy (AFM)**

Sufficient coverage of the ultrathin films on QCM-D sensors was verified by AFM using a NanoTA
AFM+ instrument (Anasys Instruments, Bruker, MA, USA) with Mounted Standard Silicon
Tapping Mode Probes with Al Reflex coating (Applied Nanostructures Inc., CA, USA). Images of the ultrathin film surfaces were recorded in tapping mode in the air with a scan rate of 0.5 Hz with silicon cantilevers. The damping ratio was around 0.7-0.85 Hz. For each sample, three different areas were analyzed, and the images were not processed by any other means except flattening. AFM images of all ultrathin film surfaces and their height profiles are presented in Supplementary Fig. 4.

**Water Contact angles (WCA)**

WCAs for ultrathin films were determined to assess the films’ chemical nature. We used a sessile drop method with a video camera-based fully computer-controlled contact angle meter (Attension Theta Optical Tensiometer, Biolin Scientific, Finland). A droplet volume of 2 µl (Milli-Q water) and a recording time of 120 s were used to measure the contact angle of the ultrathin films. 2-3 droplets were applied on the ultrathin film surfaces, and the average contact angles were calculated from these. The values used for the calculations were from time point 1.4 s. The value was taken from the beginning of the measurement since the droplet is affected by evaporation due to its small size. WCA values are shown in Supplementary Fig. 3 for all ultrathin films on the upper part of the AFM image.

**Quartz Crystal Microbalance with Dissipation (QCM-D)**

Adsorption of PS particles (LB1, LB11) on native CNF, TEMPO-CN, RC, and PS ultrathin films was investigated using E4 QCM-D instrument (Q-Sense AB, Gothenburg, Sweden). QCM-D is used for following *in situ* changes of mass at solid/gas and solid/liquid interface, since the measured change in frequency ($\Delta f$) corresponds to the change in areal mass ($\Delta m$). Simultaneously the change in dissipation ($\Delta D$) is monitored yielding information on the changes in viscoelastic properties of the adsorbed layer. The interpretation of QCM-D data is described elsewhere in detail. If the adsorbed film is evenly distributed and rigid, the change in frequency is directly proportional to the
change in areal mass and can be calculated according to the Sauerbrey relation presented in Equation 1\(^45\).

\[
\Delta m = - \frac{C}{n} \Delta f
\]

where \(\Delta m\) is the areal mass, \(n\) is the overtone number \((n = 1, 3, 5, 7, 9, 11)\), and \(C = 17.7 \text{ ng (cm}^2\text{ Hz}^{-1}\)) for the 5 MHz AT-cut crystal. Changes in dissipation must remain low \(<10^{-5}\) for the Sauerbrey equation to remain valid. Larger changes indicate a softer and thicker layer, where the amount of adsorbed water is significant.

PS particle adsorption experiments were carried out as follows. Purified and stable 0.1 g L\(^{-1}\) PS particle \((\phi = 100 \text{ nm, nP})\) dispersions were prepared in phosphate buffer (10 mM, pH 6.8). QCM-D sensor surfaces coated with ultrathin films were stabilised in the buffered conditions for at least 12 hours. Before introducing PS particle dispersion to the QCM-D chamber, the sensor surfaces were contacted with the phosphate buffer solution for approximately 1 hour to avoid the bulk effect. Then 0.1 g L\(^{-1}\) PS particle dispersion was introduced into the QCM-D chamber with the flow rate of 0.1 ml min\(^{-1}\) for approximately 1 hour. The particle adsorption was confirmed by rinsing the surface with phosphate buffer for 1 h. Two replicates of each measurement were performed. The adsorption of PS particles, as well as the possible desorption due to rinsing were monitored by following the changes in frequency \((\Delta f)\) and dissipation \((\Delta D)\) as a function of time.

**Scanning Electron Microscopy (SEM) and image analysis**

Self-standing and ultrathin films were imaged after fluorescence and QCM-D measurements with a Merlin Field Emission (FE)-SEM (Carl Zeiss NTS GmbH, Germany) to visualize the films after particle capture and adsorption. The self-standing films and QCM-D crystals were dried after the measurements and attached onto SEM sample holders using carbon tape. Samples on the holders
were coated with gold by sputtering (2 nm thick gold surface) to improve sample conductivity. Samples were imaged with the electron gun voltage of 3-5 kV and the grid current of 60 pA. The number of pixels in the SEM image was 2048 (H) x 1536 (V), with 256 gray levels. At least three SEM images of each sample were acquired at different positions. In addition, the number of adsorbed PS particles on the ultrathin films was quantified using image analysis, which was developed to recognize the PS particles in SEM images to determine the particle amount per mm$^2$.

In SEM imaging, as in imaging methods general, images are clipped within a rectangular boundary. When a spatial pattern is observed through a rectangular clipping window, several edge defects arise. One of these edge defects is size-dependent sampling bias. Miles has discussed plus-sampling (any object that intersects the clipping window is accepted) and minus-sampling (only those objects that lie within the clipping window are accepted). In our research, no attempt was made to determine the particle size distribution as the particles in the image were the same size. Therefore, size-dependent sampling bias was not a problem in our analysis. The relationship between the actual dimensions of the particles (μm) and the pixel size of the particles was obtained from the scale bar in the SEM image. The SEM images were of good quality; the background variation was small and bright objects (particles) stood out clearly from the dark background. Therefore, no image preprocessing was required, and the first image processing operation was segmentation. It was possible to use a global threshold value because the background variation was small. The thresholding method used in this study was based on histogram shape information. The threshold was chosen for the descending part of the prominent peak of the histogram (see Supplementary Fig. 9). The aim was to identify the individual PS particles and their centers, making it possible to determine the total number of particles. In most SEM images, the particles were detached from each other or formed only small clusters. However, in some cases, the particles had a strong tendency to form clusters (Examples of SEM images with identified particles shown in Supplementary Fig. 8). Thus, the next step of the image analysis was divided into two methods depending on which of the
above categories the image was classified into. When the particles were mainly detached, the
particles were identified from the threshold image by their area (we know the diameter of particle
size in each image) and shape (circular objects). The size of the clusters observed was assumed to
be three particles. In samples where the clusters were large, and the particles were mainly in the
clusters, individual particles were not reliably identified. In this case, the area of each cluster was
determined, and the number of particles needed to achieve the same area was calculated. Finally,
the identified particles were presented by drawing a marker on the original SEM image (see
Supplementary Fig. 8, 9).

**Theoretical maximum adsorption of particles - Fittings with RSA model**

We used random sequential adsorption (RSA) model to evaluate the maximum adsorption capacity
of the ultraThin films. The thickness of the ultraThin film is well below 100 nm. Thus, the PS
particles cannot penetrate the film. If the goal is to determine the maximum number of PS particles
that can fit on the surface of ultraThin films, the question can be simplified to the packing of circular
disks in a plane. Adsorption of particles on solid, flat surfaces is often an irreversible process, as
was also verified by QCM-D measurements in this study (Supplementary Fig. 6). In addition,
particles usually do not adsorb one on top of each other, instead they form a monolayer
(Supplementary Fig. 7). The basic RSA model assumes that only steric repulsion is present between
the circular disks. For circular disks of the same size, saturation occurs at a surface coverage \( \theta_\infty \) of
0.547. If there are disks of different sizes (particles of varying diameter) in the system, higher
surface coverage \( \theta \) can be reached. In this study, all particles were the same size. If the viewing area
is one \( \text{mm}^2 \) and the PS particles are the same size, the maximum area covered by the particles is
0.547 mm\(^2\). In this case, a 1 \( \text{mm}^2 \) area can hold \( 5.76 \times 10^7 \) circles (PS particles) with an effective
diameter (diameter that perceives also the estimation of electrical double layer and hydration shell
of the particle) of \( d_{\text{eff}} = 1.1d_{\text{abs}} = 110 \text{ nm} \). The cross-sectional area of one particle was calculated
using \( A_{\text{particle}} = \pi(d_{\text{eff}}/2)^2 \). Also the RSA model assumes that particles hit the surface at the same rate
throughout the adsorption process. Therefore, the concentration $c$ must be high enough to form a monolayer in the saturation regime. If there are not enough particles, the adsorption process may stop before reaching the saturation surface coverage. Table 1 shows that the maximum surface mass density ($\Gamma_{\text{max}}$) was 1890 ng cm$^{-2}$ for purified nPp adsorbed on RC. With a QCM-D sensor diameter of 9 mm, there was ~1200 ng of nPp on the sensor surface. This corresponds to approximately 0.2% of nPp (100 000 ng cm$^{-3}$ nPp dispersion was introduced into the QCM-D chamber with a flow rate of 0.1 ml/min for approximately 1 hour). Therefore, we can assume that nPp hit the surface at the same rate throughout the adsorption process, and the requirements to utilize the RSA model are met. In our system, all of the main RSA principles are valid, and therefore, the adsorption behaviour of nPp particles can be described using the random sequential adsorption (RSA) model.$^{31}

Assessment of particle adsorption kinetics and the amount of coupled water

In order to evaluate the kinetics of the PS particle adsorption process, we modeled the QCM-D data (Fig. 3a, Supplementary Fig. 6) with the RSA model (Fig. 3b) with input value for number of particles gained from the image analysis. The kinetics of adsorption of particles can be described by equation 2.$^{48-50}$

$$\frac{dN(t)}{dt} = k_a c' B(\theta) - k_d \theta$$  \hspace{1cm} (2)

Where $N(t)$ is the number density of adsorbed particles (in units of 1 cm$^{-2}$), $\theta$ is the surface coverage $\theta = [0,1]$, $t$ is the adsorption time (s), $k_a$ is the adsorption rate coefficient (cm s$^{-1}$), $k_d$ is the desorption coefficient (cm s$^{-1}$), $B(\theta)$ is the surface blocking function, and $c'$ is the particle number concentration (no. cm$^{-3}$). The adsorption of polystyrene particles on a solid surface is often an irreversible process, which can be described using the RSA model ($k_d = 0$). According to Shaaf and Talbot$^{31}$, the surface blocking function $B(\theta)$ in the RSA model can be expressed as
\[ B(\theta) = 1 - 4\theta + \frac{6\sqrt{3}}{\pi} \theta^2 + \left( \frac{40}{\pi\sqrt{3}} - \frac{176}{3\pi^2} \right) \theta^3. \] (3)

The surface coverage \( \theta \) can be represented by

\[ \theta = \frac{\Gamma a}{m}, \] (4)

where \( a \) is the occupied area of a single particle (cm\(^2\)), \( m \) is the mass of a single particle [ng], and \( \Gamma \) is the adsorbed mass per unit area (ng cm\(^{-2}\)). Equation 3 is valid for \( \theta < 0.3 \). At higher surface coverage, the kinetics can be approximated with equation 5. Near the jamming limit (\( \theta_{\infty} = 0.547 \)), \( K_0 \) is about 8.98.

\[ B(\theta) = K_0(\theta_{\infty} - \theta)^3 \] (5)

Noting that \( \Gamma(t)=N(t)m \), \( c=c'm \) (\( c \) in units of ng cm\(^{-3}\)), and combining equations 2, 3, and 4 we get,

\[ \frac{d\Gamma(t)}{dt} = k_a c \left( 1 - 4\frac{\Gamma}{m} + \frac{6\sqrt{3}}{\pi} \left( \frac{\Gamma}{m} \right)^2 + \left( \frac{40}{\pi\sqrt{3}} - \frac{176}{3\pi^2} \right) \left( \frac{\Gamma}{m} \right)^3 \right). \] (6)

The goal was to determine the adsorption coefficient \( k_a \) (cm s\(^{-1}\)) and the occupied area per nPp \( a \) (cm\(^2\)). Supplementary Fig. 5a shows the frequency change determined by QCM-D for the adsorption of stable nPp from a solution with a concentration (\( c \)) of 0.1 g L\(^{-1}\) (100 000 ng cm\(^{-3}\)) onto different surfaces. Adsorbed mass was calculated using Equation 1. All analysis was based on the 5\(^{th}\) overtone (25 MHz, \( f_0 = 5 \) MHz, \( n = 5 \)). The RSA fittings were performed using Matlab’s Curve Fitting Toolbox. Equation 6 was the custom equation. Trust-Region algorithm was used with the following coefficient starting points \( k_a c = (a/m) = 0.1 \). Lower and upper bounds for both coefficients were 0 and 10. Because QCM-D measurement detects both water and nanoplastic particles adsorbed on the sensor surface, QCM-D was combined with a direct observation method (SEM imaging coupled with image analysis) to obtain the adsorbed dry mass. The amount of adsorbed nPp was calculated from SEM images of dry ultrathin films after the adsorption.
measurements. SEM images were then analyzed as described above, and the number or particles was determined. Since the mass of a single nanoplantastic particle was known ($5.26 \times 10^{-7}$ ng) the total dry mass can be calculated ($\Delta m_{IA}$) which equals to the surface mass density $\Gamma$ (ng cm$^{-2}$). To eliminate the influence of water on QCM-D measurement, the QCMD-D data m(t) were rescaled utilizing the surface mass density ($\Gamma$) determined from the SEM images ($\Gamma(t) = m(t) \times (\Gamma_{max}/m_{max})$) where max refers to the maximum value determined by each measurement method.

To estimate the amount of coupled water (water strongly interacting with the nPp particles) the total dry mass of particles at the end of the adsorption process (analysed via image analysis) was first translated into theoretical frequency change ($\Delta f(IA)$) by applying the Equation 1. Secondly, the true frequency changes ($\Delta f(QCM)$) obtained from the QCM-D measurements were compared with the theoretical frequency changes to reveal the amount of adsorbed water as exemplified in the following calculation:

Example calculation for determining the theoretical frequency response for CNF surface with adsorbed purified 100 nm particles for the 5th overtone and comparing it to the non-normalized frequency response from the QCM-D measurement.

$$\Delta f_5(IA) = \frac{-\Delta m_{IA} \times n}{C} = \frac{419 \frac{ng}{cm^2} \times 5}{17.7 \frac{ng}{cm^2 \times Hz}} = -118.5 \text{ Hz}$$

$$\frac{\Delta f_5(QCM)}{\Delta f_5(IA)} = \frac{-373.5 \text{ Hz}}{-118.5 \text{ Hz}} = 3.15 \ldots \approx 3.2$$

For CNF and RC surfaces and both particle types (purified and stable) the frequency response was a factor of 3 larger for the experimental frequency compared to the calculated frequency from image analysis. This result indicates that 2/3 of the sensed mass by the QCM-D 5th overtone frequency corresponds to the coupled water. For PS ultrathin film, the result was that 3/4 of the
sensed mass by QCM-D was coupled water. According to previous studies, the difference between
the hydrated and dry mass is typically a factor of 1.5-40\(^{32}\). The factor in the case of adsorbing nPp is
in the lower range since QCM-D is typically used to study protein adsorption, where the proteins
can be thought as soft spheres, including water in their structure. In contrast, in the case of nPp they
are hard spheres with water only on the surface. Thus, the amount of water that adsorbs along the
particles is smaller than for proteins.

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Author contributions

IL performed the QCM-D experiments and SEM imaging for image analysis. IL performed the
sample preparation and necessary characterizations for all measurements. IL contributed to the
interpretation of all data and wrote the first version of the manuscript with the co-authors and
participated in finalizing it. TL (VTT) conducted the image analysis and RSA fittings, interpreted
RSA data and wrote the respective parts. TL (Aalto/VTT) executed the fluorescent experiments
with self-standing films. CJ executed the fluorescent microscopy experiments with hydrogels. SA
planned and supervised experimental work of IL, TL, and CJ. SA interpreted and handled
fluorescent and QCM-D data. SA wrote the first version of the manuscript with the co-authors and
prepared the figures. TT initiated the research concept, contributed to the experimental planning,
supervised the interpretation of all data and finalized the manuscript. All authors approved the
manuscript.
Competing interests

The authors declare no competing interests.

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