Protein-assisted 2D assembly of gold nanoparticles on a polysaccharide surface†

Laura Taajamaa,a Orlando J. Rojas,a,b Janne Laine,a Kirsi Yliniemi,c and Eero Kontturi*a

Site-specific assembly of gold nanoparticles on a polysaccharide surface was accomplished via a straightforward method exploiting interfacial polymer blends, selective protein adsorption and electrostatic interaction. The method could be useful in further applications due to the universal nature of the utilized phenomena.

Site-specific assembly of molecular and supramolecular architectures on 2D systems is a fundamental challenge.¹ Potential uses of such systems include biomedical applications, diagnostics, data storage, nanoreactors and sensors.¹,² The 2D realm is ever more intriguing when nanoparticles (NPs) are incorporated in these systems, and as a result numerous methods have been published to explicitly decorate surfaces with various NPs.³ These techniques are often template-based, relying on self-assembly and/or lithography.¹ In addition, organization at a liquid–air interface (Langmuir film) has been extensively used in the construction of 2D NP assemblies.⁴

Although numerous accounts exist on arranging NPs on polymer surfaces, we are not aware of any studies that present site-specific NP assemblies on flat polysaccharide surfaces. On the other hand, 2D oligo- and polysaccharide systems are an active field of research²,⁵ with applications in, e.g., analytics,²,⁵,⁶ drug release⁷ and cell encapsulation.⁸ Moreover, macroscopic chemical patterning of paper, which is essentially a network of polysaccharide-based fibres, has recently gained attention.⁹ Clearly, a facile method to immobilize NPs on flat polysaccharide surfaces with micro- and nanoscale lateral precision is required. Here, we present the 2D assembly of gold NPs (AuNPs) on specific, micro- and nano-sized sites on an ultrathin polysaccharide film. The method is based on simple physicochemical phenomena: (i) interfacial phase separation of immiscible polymer blends, (ii) site-specific adsorption of bovine serum albumin (BSA) protein on polystyrene (PS) on an ultrathin cellulose film, and (iii) electrostatically driven adsorption of anionic AuNPs on BSA (Fig. 1). The NP textures range from cellular networks to circular patches on cellulose. It is precisely the well-established nature of all these three phenomena that enables the effortlessness of their combination. The current trend towards sustainable solutions promotes the use of renewable, biodegradable and non-toxic cellulose materials. Furthermore, cellulose has the potential for the construction of various 2D architectures.¹⁰ Cellulose films were fabricated by spin coating immiscible PS and trimethylsilyl cellulose (TMSC) blends on silicon crystals followed by selective conversion of the TMSC to cellulose [Fig. S1, ESI†], which does not affect the PS domains in any way. Although hydrophilic, cellulose does not dissolve in water and these films have been shown to be stable in aqueous environments.¹⁰,b,c Both protein and AuNP (average diameter ca. 20 nm) adsorption was followed with a Quartz-Crystal Microbalance with Dissipation monitoring (QCM-D) instrument. Schematic of the adsorption process is shown in Fig. 1. Further details of the experimental work performed are described in ESI.†

Fig. 2 shows the course of the BSA and AuNP adsorption on the cellulose films. In QCM-D, increasing adsorbed mass is
detected as a decrease in frequency of the oscillating crystal. Although most proteins exhibit moderate adsorption on cellulose, BSA does not adsorb on cellulose domains, whereas the well-reported adsorption of proteins on PS occurs abundantly.

The lack of BSA adsorption on cellulose is also demonstrated by the QCM-D data: pure cellulose film (PS–cellulose ratio 0 : 1) does not trigger any response from the resonance frequency, indicating negligible adsorption of BSA and, subsequently, AuNPs. As expected, the more PS in the film the higher the amount of adsorbed BSA and consequently AuNPs (Fig. 2). Proteins are versatile substrates for electrostatically driven adsorption because of their zwitterionic nature which enables the tuning of their charge sign by simply adjusting pH. Thus, attachment of AuNPs carrying a negative charge was achieved by adjusting the pH to 4.5, i.e., below the isoelectric point of BSA (ca. pH 5). The pH level was tuned to this value because we need to avoid the protonation of carboxylic acid moieties protecting the AuNPs, which would occur at their pKa value of approximately 4. Such a narrow pH window did not allow for tuning the amount of AuNPs, which may be feasible in other types of systems. The adsorbed BSA or AuNPs were not removed upon rinsing with their respective buffer solution nor with pure water. QCM-D results were quantified by calculating the adsorbed masses (Table S1, ESI†) and complemented with Atomic Force Microscopy (AFM) (Fig. 3, Fig. S3 and S4, ESI†) and X-ray Photoelectron Spectroscopy (XPS) measurements (Table S3, ESI†). Furthermore, the dissipation data obtained from QCM-D were used to evaluate the viscoelastic properties of the adsorbed layers. The data revealed that adsorbed BSA and AuNPs were rigidly bound (Fig. S2, ESI†).

Representative AFM height images of the modified PS–cellulose surfaces are shown in Fig. 3. Previously, it has been demonstrated that the blend ratio in the initial spin coating solution can be used to linearly tailor the amounts of the blend components in the PS–cellulose films. Surface morphology and the amounts of assembled AuNPs were tunable by simply adjusting the polymer blend ratio in the films. Morphologically, the structures decorated with AuNPs ranged from cellular network structures (Fig. 3e) to discrete circular patches (Fig. 3d) on cellulose surfaces. Cellular networks with AuNPs have aroused considerable interest recently because of their potential in electronic applications. The pure cellulose film was smooth with only a negligible amount of AuNPs present (Fig. 3a), while the pure PS film was entirely decorated with AuNPs (Fig. 3f). Phase separation in the case of the PS–cellulose blend ratio of 2 : 5 was not as complete as with the other blend ratios which can be regarded as the reason behind ostensibly non-selective AuNP assembly (Fig. 3e). Previous studies on interfacial spin coated blends with synthetic polymers have shown that with close to equal blend ratios, phase separation is far from complete. Hence, with the 2 : 5 ratio, the PS and cellulose phases are not entirely unalloyed, but contain traces of the other component enabling BSA and consequent AuNP adsorption within the cellulose phase as well.

The quantitative analyses of attached AuNPs from QCM-D, AFM and XPS experiments coincide well (Fig. 4). The amounts of

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**Fig. 2** AuNP assembly on polysaccharide surfaces followed with a QCM-D: change in frequency (Δf) as a function of time. The PS–TMSC blend ratios from initial spin coating solutions are indicated on the right. Blend ratios 0 : 1 and 1 : 0 correspond to cellulose and PS, respectively.

**Fig. 3** The morphology of the modified polysaccharide surfaces: 3D AFM height images (5 × 5 μm²) of the PS–cellulose films after BSA and AuNP adsorption. The PS–TMSC blend ratios from initial spin coating solutions are stated on top of each image. Blend ratios 0 : 1 and 1 : 0 correspond to cellulose and PS, respectively.

**Fig. 4** Comparison of quantitative information acquired from QCM (■, solid line), AFM (▲, dashed line) and XPS measurements (●, dotted line). PS–TMSC blend ratios (w/w) in the initial spin coating solutions are stated on the bottom. Blend ratios 0 : 1 and 1 : 0 correspond to cellulose and PS, respectively. Inset: adsorbed amounts of BSA for the same blend ratios as deduced from QCM-D data. The lines are added to guide the eye.
AuNPs also correlate with the adsorbed amounts of BSA as shown in the inset of Fig. 4, which also indicates the total lack of adsorption of BSA on pure cellulose. The data in Fig. 4 are also in agreement with previous studies on PS containing polymer films and protein adsorption where it was concluded that increasing the amount of hydrophilic component was found to hinder protein adsorption.13,14 Protein adsorption was demonstrated to be governed largely by hydrophobic effects.15 As mentioned above, the amount of adsorbed AuNPs in the blend surfaces increased with increasing proportion of PS (Fig. 4). The discrepancy in the case of the 2 : 5 ratio arises from the fact that the domain sizes were larger and phase separation was less complete than in the case of the other blend ratios. This resulted in fewer preferred adsorption sites16 for the protein and is hence postulated to be accountable for the smaller amount of detected AuNPs. It must be emphasized that the amount of adsorbed AuNPs depends also on, for example, the ionic strength, concentration, charge density of the substrate and the nanoparticles, and temperature, but their effect on the adsorption is out of the scope of this communication. However, when these parameters are retained constant, the amount of PS governs the amount of adsorbed AuNPs as demonstrated in Fig. 4.

None of the phenomena that form the basis of the present method are new but it is precisely their familiarity that enables the utilization of this method as a platform technology for different nanoparticle arrays on polysaccharide surfaces. Because of the universal nature of interfacial phase separation of polymers, protein attachment on PS and electrostatically driven adsorption, we envisage that the presented method as a platform technology for attaching charge-stabilized NP textures on polysaccharide surfaces. Hypothetically, many kinds of systems are feasible. For example, since the charge of the zwitterionic polymer may be tuned with a mere pH adjustment, both cationically and anionically stabilized particles can be applied (data for the feasibility of adsorbing cationic AuNPs on anionic BSA can be found in Fig. S5, S6 and Table S2, ESIF). Moreover, since 2D structures can, at times, be converted to 3D systems, a similar approach could be used to build multi-component, structurally well-defined 3D frameworks from cellulose as well as other polysaccharides with various nano-objects. These structures are bound to bear a higher degree of precision than the current bulk assemblies of polysaccharides and NPs.16 An additional asset of this system is the prospect of obtaining cellular network style architectures for, e.g., charge transport applications,12 which may facilitate the use of these textures in protein- and polysaccharide-related analytical applications. Furthermore, utilization of, for example, click-activated or modified proteins could open up novel routes for engineering diverse polysaccharide surfaces.17

We acknowledge Dr Joseph Campbell and Dr Leena-Sisko Johansson for recording the XPS data and helping in analysing the results. We are grateful to Tekes – the Finnish Funding Agency for Technology and Innovation for support to the LignoCell project. L.T. acknowledges Hannes Orelma (MSc) and Karoliina Junka (MSc) for scientific discussions and Katri Konturi (MSc) for additional experimental work. E.K. and K.Y. acknowledge Academy of Finland (Projects 259500 and 263551, respectively) for financing.

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ELECTRONIC SUPPLEMENTARY INFORMATION

for

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by

Laura Taajamaa, Orlando J. Rojas, Janne Laine, Kirsi Yliniemi, Eero Kontturi

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- Detailed description of the experimental work (Chapter Experimental details)
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- QCM-D modelling results (Table S1)
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Experimental details

Materials
Trimethylsilyl cellulose (TMSC) (Fig. S1) was synthesized and characterized as described previously.1, 2 Weight average molecular weight and number average molecular weight obtained from size-exclusion chromatography measurements were ca. 227000 and 79000 g/mol, respectively, and polydispersity index (PDI) was 2.9. The degree of substitution based on XPS results was 2.1. Polystyrene (PS) (Aldrich 182427) had molecular weight of 280 000 Da according to the manufacturer.

Albumin from bovine serum (BSA) was commercial product from Sigma-Aldrich (A7030). According to the manufacturer, purity was ≥98 %, the product was essentially fatty acid and globulin free and molecular weight was ca. 66 kDa. Gold nanoparticles (AuNPs) were purchased from Sigma-Aldrich (741965). The diameter was 20 nm, PDI<0.2, core size 18-22 nm and mean hydrodynamic diameter 28-36 nm, according to the manufacturer. Poly(allylamine hydrochloride) (PAH, Mw≈58 000, Aldrich) was used for depositing a cationic layer on commercial, anionic
AuNPs. HCl (Aldrich) was used to control the pH of the solution prior to the zetapotential measurements, if necessary.

All solvents and other chemicals were of analytical grade and used as obtained from the manufacturer. Water used was of ultra-high quality (UHQ) (resistivity 18.2 MΩ cm) purified with Millipore Direct-Q® 3UV (Millipore, Molsheim, France).

**Methods**

**Spin Coating.** Ultrathin films of PS and TMSC blends (here referred as PS/TMSC surfaces) were prepared by spin coating. TMSC and PS were separately dissolved in toluene with solution concentration of 10 g/dm³. Blends were prepared by mixing the two 10 g/dm³ solutions and diluting the rest with toluene in a way that the majority component concentration was always 5 g/dm³ and the minority component concentration varied according to the PS/TMSC ratio. Pure cellulose and PS films were prepared from 5 g/l solution to obtain the same amount of polymer as in the blends. The spin coater used was WS-650SX-6NPP/LITE (Laurell Technologies Corporation, North Wales, PA, USA). AT-cut silicon dioxide QCM-D crystals (BiolinScientific, Västra Frölunda, Sweden) were used as substrates. Before mounting the substrates on the spin coater, they were cleaned in the UV/ozonator for at least 20 minutes and prior to spin coating the bare crystals were rinsed twice with toluene (4000 rpm for ca. 10 s). Spin coating was performed with the spinning speed of 4000 rpm and with the acceleration speed of 2130 rpm/s. The deposition of the solution was performed on a static substrate and the spinning was retained ca. 30 seconds after the disappearance of the Newtonian rings which usually took place already during the acceleration.

**Selective conversion of TMSC to cellulose.** The spin coated films containing TMSC (PS/TMSC surfaces) were hydrolysed in a 2 M aqueous HCl vapor environment under vacuum for 2 minutes to obtain PS/cellulose films. During the hydrolysis, TMSC is converted back to cellulose thus the TMSC patches contract due to the replacement of the bulky trimethylsilyl groups with tightly packed hydroxyl groups (Fig. S1).

![Fig. S1. Selective conversion of TMSC (bottom) to cellulose (top).](image)

**Preparation of cationic nanoparticles (AuNP-PAHs).** Cationic AuNPs were synthesized by attaching electrostatically a cationic PAH layer on the top of the protective anionic layer of commercial AuNPs (i.e., utilizing layer-by-layer method). 4 ml of anionic AuNPs were shaken with 4 ml of 2 mg/ml PAH solution for 20 min. The formed AuNP-PAHs were centrifuged to remove the
excess of PAH solution, washed with water, centrifuged again and then re-dispersed into 1 ml of water.

**Zetapotential measurements of AuNP-PAHs.** Zetapotential measurements were performed with dynamic light scattering instrument (Zetasizer Nano series, Malvern Instruments Ltd) at 25°C, using a disposable zetapotential cell (folded capillary cells, Malvern Instruments Ltd). Data was analysed by the Zetasizer software (Malvern Instruments Ltd) and using literature values of bulk Au for the refractive index and absorption at 633 nm (n=0.180, a=0.120).³⁴ The zetapotential of commercial, anionic AuNPs was measured prior to mixing with PAH solution and it was -20 mV (pH=6.5). Subsequently, to prove the formation of cationic AuNPs after AuNP-PAH synthesis, the zetapotential of Au-PAHs was measured and it was +39 mV (pH = 8.9). As PAH is a weak polyelectrolyte and its degree of ionization strongly depends on pH, the cationic nature of AuNP-PAHs was ensured also in the measurement conditions by determining zetapotential of AuNP-PAHs at pH = 7.5 and it was +22 mV.

**AuNP assembly on polysaccharide surfaces using Quartz-Crystal Microbalance with Dissipation monitoring (QCM-D).** Protein-assisted AuNP assembly was performed with a Q-Sense E4 instrument (Q-Sense AB, Gothenburg, Sweden). The crystals with PS/cellulose surfaces were immersed overnight in water and thoroughly dried with nitrogen gas. After the crystals had been mounted to the measuring chamber in QCM-D instrument, a continuous flow of dilute (≤0.01 μM) ammonium hydroxide solution was introduced to the chamber. The pH of ammonium hydroxide was adjusted close to eight. When a stable baseline was reached, the measurement was started and after ten minutes, BSA dissolved in dilute (≤0.01 μM) ammonium hydroxide solution was presented into the chamber. The pH of the 0.1 g/dm³ BSA solution was the same as that of the pure dilute ammonium hydroxide. The isoelectric point (pI) of BSA is ca. at pH 5 thus during adsorption, it carries a negative net charge. Also cellulose surface has weak negative charge.⁵ The BSA adsorption continued for 45 minutes and it was followed by a rinsing step with ammonium hydroxide and sodium acetate-acetic acid (NaAc/HAc) buffer (pH ~4.5) to remove any unbound BSA and to adjust BSA charge from anionic to cationic. Electrostatic attachment of citrate-stabilized AuNPs carrying negative charge into BSA patches was achieved by adjusting pH from ca. 8 to 4.5 with NaAc/HAc buffer. After the protein adsorption and rinsing steps, the 3.4 ng/dm³ AuNP solution in NaAc/HAc buffer (pH ~4.5) was introduced and adsorption was continued for 30 minutes. In the case of cationic nanoparticles (AuNP-PAH), the pH during the adsorption was adjusted to 7.5 in order to retain BSA anionic. The surfaces were then rinsed with NaAc/HAc buffer (in the case of the anionic AuNPs only) and UHQ water (in the case of both anionic and cationic AuNPs) to remove any excess AuNPs. After the stabilization of the frequency and dissipation curves, the measurement was stopped. All measurements were recorded at 5 MHz fundamental resonance frequency and its overtones 15, 25, 35, 55 and 65 MHz and with constant 0.1 ml/min pump flow rate. Only the fifth overtone (25 MHz) is displayed in figures for clarity. The temperature was stabilized to 23°C and each experiment was repeated at least two times. The QCM-D results were analysed quantitatively with Voigt model explained in more detail below.

**Atomic Force Microscopy (AFM).** Surface morphology was characterised using MultiMode 8 scanning probe microscope (Bruker AXS Inc., Madison, WI, USA). The images were scanned in tapping mode with E scanner and silicon cantilevers (NSC15/AIBS from Ultrasharp μmasch,
Tallinn, Estonia). The radius of curvature for the tip according the manufacturer was less than 10 nm and typical resonance frequency of the cantilever was 325 kHz. At least two parallel surfaces were prepared and at least two points on each were imaged. No image processing besides flattening was performed. All quantitative data was extracted from the height images.

**Image Analysis** was performed using Nanoscope Analysis (version 1.20, Veeco, Plainview, NY, USA) and Scanning Probe Image Processor (SPIP) (version 6.0.2, Image Metrology, Lyngby, Denmark) softwares. The surface coverage of AuNPs was determined from $5 \times 5 \mu m^2$ height images using the Particle and Pore analysis module in SPIP with Threshold algorithm. The Z-scale height parameter in 3D AFM images (Fig. 3) was adjusted to 50 nm and aspect ratio to 8.

**X-Ray Photoelectron Spectroscopy (XPS).** Chemistry of the surfaces was studied with XPS. XPS measurements were performed with an AXIS 165 (Kratos Analytical, Manchester, UK) spectrometer using a monochromated Al Kα X-ray source. All samples were pre-evacuated overnight to stabilize ultra-high vacuum (UHV) conditions. UHV condition was monitored during the whole measurement. Two parallel samples were prepared and each sample was analysed at three points. Elemental surface composition was determined from low resolution scans recorded with 80 eV pass energy and 1 eV steps. Relative amounts of nitrogen and gold were obtained from N 1s and Au 4f regional scans recorded with the same resolution and step intervals as survey scans, only with extended acquisition times. Carbon 1s and oxygen 1s high resolution spectra were determined using 20 eV pass energy at 0.1 eV steps. The carbon 1s emission was resolved into various contributions corresponding to distinct chemical states of carbon according to literature.6

**Size-exclusion chromatography (SEC).** The molecular weight of TMSC was determined with SEC using chloroform with 2% triethylamine (TEA) as eluent. Elution speed was 1 mL/min through the following column system: PLgel precolumn and PLgel, 104, 105, 103, and 102 Å columns supplied by Polymer laboratories. Relative changes in molecular weight were determined with a Waters RI-detector (refractive index) against polystyrene standards at 35 °C.

**Additional results**

**QCM-D Dissipation data.** QCM-D provides information not only on the adsorbed amount but also on the viscoelastic properties of the adsorbed layer via dissipation monitoring. Dissipation data is obtained by stopping the driving voltage and recording the decaying amplitude of the crystal as a function of time. Rigid layers have low dissipation values while more viscoelastic layers exhibit higher values due to short damping times caused by greater frictional losses. The dissipation changes as a function of time are shown in Fig. S2. Only minor changes in dissipation indicate that the adsorbed BSA and AuNPs were rigidly bound and did not swell or bound water significantly. Also, no major differences between the PS/cellulose blend ratios were noted. This is understandable since all the films that have cellulose as a majority component (PS/TMSC 0:1, 1:50, 1:20 and 1:5) contain the same amount of cellulose (5 g/dm³ in the initial spin coating solution) and cellulose is the component that swells and binds water in these ultrathin films.
Fig. S2. AuNP assembly in QCM-D: changes in dissipation (ΔD) as a function of time. The label size increases with the increasing amount of PS in the film (from blend ratio PS/TMSC 0:1 to 1:0 films).

**QCM-D Data Modelling.** When the adsorbed component is evenly distributed and rigidly bound to the surface, and its mass low enough compared to the mass of the crystal, the adsorbed amount can be estimated with Sauerbrey’s equation

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

where C is the mass sensitivity constant (C = 0.177 mg/(Hz \(^{-1}\)m\(^2\)) for a 5 MHz crystal), n is the overtone number (n = 1, 3, 5...), and Δf is the observed change in frequency. If, however, the adsorbed layer does not meet the Sauerbrey conditions, the equation 1 underestimates the adsorbed mass. Voigt viscoelastic, parallel spring and damper iterative model was used to estimate the adsorbed masses of BSA and AuNPs on PS/cellulose blend surfaces. The calculations were performed with Q-Tools (version 2.1, Q-Sense, Västra Frölunda, Sweden) software. When the Voigt model is applied, certain assumptions need to be made and here 1200 g/m\(^3\) was used for the density of the adsorbed layer. The obtained values for the adsorbed BSA and AuNP masses (mg/m\(^2\)) are presented in Table S1.

<table>
<thead>
<tr>
<th>PS/TMSC blend ratio</th>
<th>BSA adsorption</th>
<th>AuNP adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1:50</td>
<td>0.28</td>
<td>1.77</td>
</tr>
<tr>
<td>1:20</td>
<td>0.12</td>
<td>1.10</td>
</tr>
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<td>1:5</td>
<td>1.17</td>
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<td>2:5</td>
<td>1.97</td>
<td>4.26</td>
</tr>
<tr>
<td>1:0</td>
<td>4.31</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Table S1. Results from the QCM-D modelling with Voigt model. The PS/TMSC blend ratios (w/w) from initial spin coating solutions are stated and blend ratios 0:1 and 1:0 correspond to cellulose and PS, respectively.

**Surface morphology evaluation.** The surface morphology of the PS/cellulose films was characterised with AFM before and after the AuNP assembly (Fig. S3). Pure cellulose stays mainly
intact during the AuNP assembly while PS film undergoes pronounced changes. BSA adsorbs selectively to PS domains in the blend films under these conditions. No major changes in the overall morphology of the blend films on a large scale were noted but once smaller scan sizes or individual height scans were observed the appearance of AuNP (diameter ca. 20 nm) was obvious. The citrate-stabilised particles seemed to stay stable also during adsorption since no major aggregates were noted in AFM images after the AuNP assembly. Also films at intermediate stage of the process (after BSA adsorption and rinsing step) were imaged but BSA seems to adsorb in such a flat conformation that no changes in the morphology were observed, even at high magnifications (Fig. S4).

**Fig. S3.** Representative 2D AFM height images before (top row) and after (two bottom rows) AuNP assembly. White and black scale bar correspond to 1 μm and 200 nm, respectively. PS/TMSC blend ratios (w/w) in the initial spin coating solutions are stated on top. Blend ratio 0:1 and 1:0 correspond to cellulose and PS surfaces, respectively. In the AFM height images, the light areas are higher and dark areas are lower. On top of the 5 × 5 μm² height images after the AuNP assembly, there are individual height scans and in the corresponding image, white line indicates the place where the height scan is taken. Note that horizontal scale accuracy is limited in AFM.
**Fig. S4.** Representative 2D AFM height images of PS/cellulose blend films before (top row) and after (three bottom rows) BSA assembly. The length of the scale bar is 200 nm in all images, i.e., the bottom three rows represent different magnifications. PS/TMSC blend ratios (w/w) in the initial spin coating solutions are stated on top. Blend ratio 0:1 and 1:0 correspond to cellulose and PS surfaces, respectively. In the AFM height images, the light areas are higher and dark areas are lower.

**Adsorption of cationic AuNPs (AuNP-PAHs).** BSA was adsorbed on a PS surface under similar adsorption conditions as described for the previous experiments. The adsorption was carried out for 20 minutes. Subsequently, the pH was tuned to 7.5 to retain the BSA anionic (isoelectric point at pH~5), and the surface was exposed to the AuNP-PAHs. Fig. S5 clearly shows that the procedure for attaching AuNP-PAHs on the BSA treated PS surface was successful: decrease in the frequency indicates an increased mass due to the adsorption of AuNP-PAHs.
The quantity of the adsorbed AuNP-PAHs was calculated from the QCM-D data of Fig. S5, according to the previously described Voigt modelling (see section QCM-D Data Modelling). Furthermore, the adsorbed amount of AuNP-PAHs was quantified by AFM imaging the surfaces and subjecting the images to image analysis as previously described. The AFM images are shown in Fig. S6, and Table S2 reveals the quantitative results from QCM-D and AFM analysis. It is evident that the amount of the adsorbed AuNP-PAHs is very similar to the values of the adsorbed anionic AuNPs (see Fig. 4 in the main article).

**Table S2.** Results from the QCM-D modelling with Voigt model and AFM image analysis from the PS films after sequential adsorption of BSA and AuNP-PAHs.

<table>
<thead>
<tr>
<th>Blend ratio</th>
<th>AuNP-PAH Voigt mass, mg/m² (QCM)</th>
<th>Particle count (AFM)</th>
<th>Coverage, % (AFM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS/TMSC 1:0</td>
<td>5.50</td>
<td>188±18</td>
<td>1.7±0.18</td>
</tr>
</tbody>
</table>

**Surface chemical composition.** XPS was used to confirm the successful AuNP assembly on the PS/cellulose films (Table S2). The relative amounts of nitrogen (N 1s) and gold (Au 4f) emissions were used as fingerprints for BSA and AuNPs, respectively, since they are not present in the films before assembly. Generally, the more PS in the films, the higher the amount of both nitrogen and gold on the surface after AuNP assembly. However, in the case of PS/cellulose 2:5 the adsorbed amounts are smaller. This is hypothesized to derive from the fact that with this blend ratio the phase...
separation was more incomplete and also the domain sizes were larger. The phase separation occurs during rapid spin coating process and the TMSC (consequent cellulose) and PS phases contain traces of the other component as well. Also it has to be noted that the quantitative XPS analysis has its limitations and prerequisite is homogenous distribution of the components on the surface.

Table S3. XPS results. The PS/TMSC blend ratios (w/w) from initial spin coating solutions are stated and blend ratios 0:1 and 1:0 correspond to cellulose and PS, respectively.

<table>
<thead>
<tr>
<th>Blend ratio</th>
<th>Atomic Concentrations</th>
<th>C 1s Hi-Res components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O 1s</td>
<td>C 1s</td>
</tr>
<tr>
<td>0:1</td>
<td>43.12±0.67</td>
<td>54.71±0.93</td>
</tr>
<tr>
<td>1:50</td>
<td>41.15±0.65</td>
<td>56.84±0.49</td>
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<td>1:20</td>
<td>40.14±0.57</td>
<td>57.58±0.50</td>
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<tr>
<td>1:5</td>
<td>34.64±0.65</td>
<td>63.60±0.49</td>
</tr>
<tr>
<td>2:5</td>
<td>31.64±0.79</td>
<td>66.90±0.79</td>
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<tr>
<td>1:0</td>
<td>6.75±0.72</td>
<td>89.91±1.48</td>
</tr>
</tbody>
</table>

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