Carboxylate Functional Groups Mediate Interaction with Silver Nanoparticles in Biofilm Matrix: Supporting Information

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1. Experimental details

Chemicals

All chemicals were purchased from Sigma-Aldrich if not stated specifically below. Nanopure water (18.1 MΩ•cm, Milli-Q) was used as a solvent.

Silver nanoparticles with a nominal primary particle size of 25 nm were produced by wet precipitation from $AgNO_3$ in the presence of carbonate or citric acid by NanoSys GmbH (Wolfhalden, Switzerland) as an aqueous suspension with a nominal concentration of 1 g/L (9.27 mM) Ag. The original suspension was kept in the dark.

Silver film preparation

Samples were prepared using unbalanced DC magnetron sputter deposition from an elemental Ag target with a purity of 5N in a UHV system at room temperature. The deposition chamber used is an ATC 1500 F sputtering system from AJA international Inc. (North Scituate, MA, USA). The base pressure before deposition was $<5 \times 10^{-7}$ Pa; during deposition the pressure was kept at 5.4 Pa using a constant Ar-flow of 16 sccm. Silver was sputtered at a power density of 5.0 W/cm^2. Prior to deposition the samples were cleaned using an rf-bias on the substrate of -250 V in flowing Ar (15 sccm) at a pressure of 0.5 Pa. Samples were grown to a thickness of 200 nm, as determined using a Brucker Dektak XT surface profilometer equipped with a diamond stylus.

Colonization of stream biofilms on artificial substrates and sampling of biofilms

Biofilms were colonized on glass microscope slides (38×26 mm, Thermo Scientific) which were placed vertically in in river Chriesbach for 21 day (on campus, Dübendorf, Switzerland, 47°24'16.8"N 8°36'41.0"E) (see references [27, 28] of the main paper). For extraction of extracellular polymeric substances, biofilms were scraped off the glass slides with a clean glass slide into 1 mL/slide $NaHCO_3$ (2 mM, pH 7.6) in a glass beaker. Extracellular polymeric substances were extracted on the same day (see below).

Field sampling of stream biofilms

Samples were taken on November 5 and 6, 2015 from six rivers in the Swiss Plateau upstream and downstream communal waste water treatment plants (sites 1-12, Supplementary Table 1, Supplementary Figure 1). Three stones of similar size from similar microenvironments in the river bed were selected at each sampling site. Biofilms were brushed off the stones with tooth brushes into 15 mL of stream water, which was sampled at the site and filtered through two layers of paper towel. The stones were washed with another 15 mL of filtered stream water. Extraction of extracellular polymeric substances was performed on the same day (see below).

Water analytics

At each field sampling site, spot measures of physical parameters were taken roughly at the same distance above ground as the surfaces of the stones sampled. Water temperature was measured with a DIEHL frigoton thermometer, flow velocity with a Schiltknecht MiniAir2 Micro anemometer (flow accuracy 1.0 % fs, 3.0 % rdg).

Water chemistry of grab samples (500 mL) taken from each sampling site and of the extracted EPS (see below) was determined as follows: Na^+, K^+, Ca^{2+}, Mg^{2+}, NO_3^{2-}, SO_4^{2-}, and Cl^- content was quantified by ion-chromatography (Metrohm 761 Compact IC, with chemical suppression for NO_3^{2-}, SO_4^{2-}, and Cl^-), with a 8 mM HNO_3/1.197 mM dipicolinic acid solution as mobile phase and a Metrosep C 6 – 250/4.0 separation column (Metrohm, Na^+, K^+, Ca^{2+}, Mg^{2+}) or a Metrosep A Supp 5 100/4 mm column (Metrohm, NO_3^{2-}, SO_4^{2-}, and Cl^-) as stationary phase. PO_4^{3-} was quantified colorimetrically (Varian Cary 50 Bio Spectrophotometer) based on the formation of molybdenum blue. Silica was determined colorimetrically based on the reduction of siliciomolybdate to silicomolybdous acid in the presence of ascorbic acid using the Autoanalyzer AA3, Bran+Luebbe (Contrec). TOC and DOC were measured with a shimadzu TOC-L CSH system. LOQ were: 2.5 mg/L Na^+, 1 mg/L K^+, 5 mg/L Ca^{2+}, 2.5 mg/L Mg^{2+}, 0.5 mg/L NO_3^{2-}, 5 mg/L SO_4^{2-}, 0.5 mg/L Cl^-, 1 mg/L H_4SiO_4, 0.5 mg/L OC.

Metal concentrations were determined by ICP-MS after microwave digestion. 0.5 mL of each sample were digested with 4 mL of 65 % HNO_3 and 0.5 mL of 30 % H_2O_2 in a microwave digestion unit (MLS ultraClave 4; 10

min at 180 °C/100 bar, 14 min at 210 °C/100 bar) and diluted 1:100 with nanopure water (18.1 MΩ•cm, Milli-Q). One sample per run contained only HNO_3 and H_2O_2 to determine the background concentration of target metals. Target metal concentrations were measured by HR-ICP-MS (Element 2 High Resolution Sector Field ICP-MS; Thermo Finnigan). The instrument was calibrated with a multi-element mass standard (Merck, 1113550100). The calibration curve for data analysis was made with the calibration standard Merck IV in the concentration range 0-20 µg/L. A reference with a concentration within the calibration range was measured every 10 samples, the calibration samples were measured every 40 samples.

Extraction, characterization, and fractionation of extracellular polymeric substances (EPS) from periphyton

The extraction procedure was performed as described previously [4, 7, 13]. The biomass was resuspended by gentle pipetting and sonication in a water bath (45 kHz 60 W, VWR Ultrasonic Cleaner) for 30 s. Fine sediment and larger biomass was allowed to settle for ~1 min, the supernatant was removed and centrifuged at 1,880·g for 10 min. Biomass was resuspended a second time in 2 mL/slide fresh solution and treated as described above. All supernatants were sequentially filtered (1 µm glass fiber [VWR], 0.45 µm polypropylene [PALL], and 0.22 µm PES [Millipore] filters). Filters were washed with nanopure water (18.1 MΩ•cm, Milli-Q) prior to use. EPS extracts were stored in glass bottles at 4 °C (0.02 % (w/v) NaN_3). All extraction steps were performed on ice, the water bath for ultrasound treatment was at room temperature.

Organic carbon and nitrogen size distribution was measured by size-exclusion chromatography – organic carbon detection – organic nitrogen detection (LC-OCD-OND) as described previously [4, 7, 13]. Samples were diluted with nanopure water (18.1 MΩ•cm, Milli-Q) right before they were measured. A size exclusion column (250×20 mm, Toyopearl TSK HW-50S) was used to separate EPS compounds. The mobile phase was phosphate buffer (24 mM, pH 6.6) and the acidification solution was phosphoric acid (60 mM, pH 1.2). The detection limit was 10 µg/L for both OC and ON. The software FIFFIKUS was used to quantify total organic carbon (TOC), dissolved (DOC), and chromatographable DOC compounds (cDOC). The chromatograms obtained from LC-OCD-OND were integrated to determine the amount of biopolymers (BP, high M_r polysaccharides and proteins), building blocks of humic substances (BB), low M_r acids (LMWA), and amphiphilic/neutral compounds (NA, alcohols, aldehydes, amino acids, and ketones).

To separate the fractions BP, BB, LMWA, and NA, a Bio-Rad 2110 fractionator coupled to the LC-OCD-OND system was employed. Fractions were taken between retention times of 30-45 min (BP), 45-49 min (BB), 49-57 min (LMWA), and 57-80 min (NA).

Synthesis of EPS stabilised Ag nanoparticles

All silver nanoparticles were formed *in situ* in the UV-vis exposure system by EPS extracted from periphyton. The UV-vis exposure system was constructed with a thermostat (Lauda RC6 RC), control stirrer module (H & P Labortechnik Variomag Telemodul 40-S) and a set of lamps (Philips Master TL5 HO 54W/865 SLV/40). The conditions used for nanoparticle synthesis were as follows: Temperature: 20 °C, Stirring: 200 rpm, Light irradiation: matching sunlight spectrum.

Nanoparticle tracking analysis (NTA)

Nanoparticle tracking analysis (NTA, NanoSight LM10 equipped with a LM14 temperature controller (NanoSight Ltd.)) was used to determine a number based particle size distribution. Each sample was directly measured three times for 60 s. All NTA videos were analyzed with the same settings in batch processing mode. Analyses that resulted in less than 200 tracked particles were not used. Videos were analyzed using the NanoSight NTA 2.3 Analytical Software (NanoSight Ltd.). Settings were as follows: Background Extract: On; Brightness: 0; Gain: 1; Blur Size: 9x9; Detection Threshold Type: Single; Detection Threshold: 15; Min track length: 10; Min Expected Size: Auto; Temperature: 23 °C; Viscosity: 0.9326.

Dynamic light scattering (DLS) and zeta potential (ZP)

Dynamic light scattering (DLS) and zeta potential (ZP) were measured with Malvern Instruments Nano ZS in polystyrene quvettes. Each sample was measured at the temperature of 25 °C three times and the average results were taken.

UV-vis light absorption

UV−vIS light absorption (190-900 nm) was recorded with a CARY 100 UV-vis spectrophotometer (Agilent Technologies) in micro quartz cuvettes. Ag NP show size- and surface-specific SPR which results in a specific light absorption spectrum.

Surface enhanced Raman spectroscopy (SERS)

Raman spectra were recorded with a Bruker SENTERRA Raman microscope with 532 nm 20 mW laser and x10 objective lens. Each sample was measured on glass slides for 180 s.

Fourier transform infrared spectroscopy (FTIR)

Infrared spectra were measured with Cary 600 Series ATR-Spectrometer (Agilent Technologies). Average of 32 measurements was taken.

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) samples were prepared by casting the solution directly onto copper grids with carbon lacey network covered with a ~3 nm thin carbon film. High-resolution TEM (HR-TEM) and selected area electron diffraction (SAED) was performed on a JEOL 2200FS microscope operated at 200 kV. Typically 0.5 s exposure time was used for recording HR-TEM images. No morphological change in particles caused by the electron beam was observed during imaging and diffraction. Analysis of the images and diffraction patterns was performed using DigitalMicrographTM.

X-ray photoelectron spectroscopy (XPS)

The samples were characterized by X-ray photoelectron spectroscopy (XPS) using a Quantum 2000 (Physical Electronics Inc.) instrument under ultrahigh vacuum (<5 x 10^{-7} Pa). Monochromic aluminium Kα X-rays with a photon energy hv = 1486.7 eV were used and data were recorded at an analyzer pass energy of 23.50 eV and a step size of 0.1 eV (for the detailed spectra) and a pass energy of 58.70 eV and a step size of 0.5 eV for the survey. Argon ions and electron neutralizers were used to compensate for surface charging.
Data were collected without sputter-cleaning and with (~2 nm removed) in order to eliminate contaminations.

Glucose assay

Glucose concentration of extracted EPS samples was determined with the High Sensitivity Glucose Assay Kit (Sigma Aldrich, MAK181) according to the procedure described by the manufacturer. In the presence of glucose, a fluorometric product is formed proportional to the glucose concentration. Of each sample, 1 and 10 µL were reacted in duplicate with the reaction mix in a final volume of 100 µL in 96 well plates. Fluorescence was measured with a Tecan plate reader (excitation: 535 nm, emission: 587 nm). Standard deviation of blanks was 0.0518 pmol/µL, detection limit was thus in the range of 0.156 pmol/µL. Spiking extracted EPS with Glucose showed no interference with the assay above detection limit.

2. Additional data

Table S1: Sampling sites along Mönchaltorfer Aa including name of the WWTP, location upstream or downstream of the WWTP, and geographic coordinates.

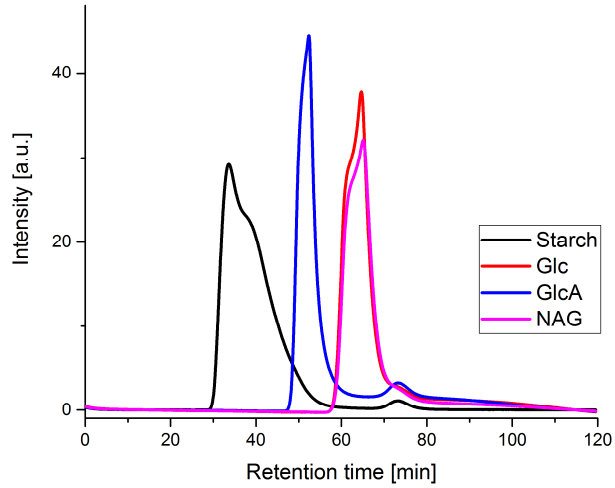
Site	Waste water treatment plant	Location	Longitude	Latitude
1	Duernten	upstream	E 8◦ 49.739	N 47◦ 16.08
2	Duernten	downstream	E 8◦ 49.809	N 47◦ 15.94
3	Moenchaltorfer	upstream	E 8◦ 44‘ 47“	N 47◦ 18‘ 10“
4	Moenchaltorfer	downstream	E 8◦ 44‘ 52“	N 47◦ 18‘ 8“
5	Elgg	upstream	E 8◦ 51.158	N 47◦ 30.049
6	Elgg	downstream	E 8◦ 51.088	N 47◦ 30.07
7	Aadorf	upstream	E 8◦ 53.596	N 47◦ 30.002
8	Aadorf	downstream	E 8◦ 53.583	N 47◦ 30.065
9	Ellikon	upstream	E 8◦ 49.624	N 47◦ 34.161
10	Ellikon	downstream	E 8◦ 49.508	N 47◦ 34.208
11	Marthalen	upstream	E 8◦ 38.391	N 47◦ 37.234
12	Marthalen	downstream	E 8◦ 38.322	N 47◦ 37.232

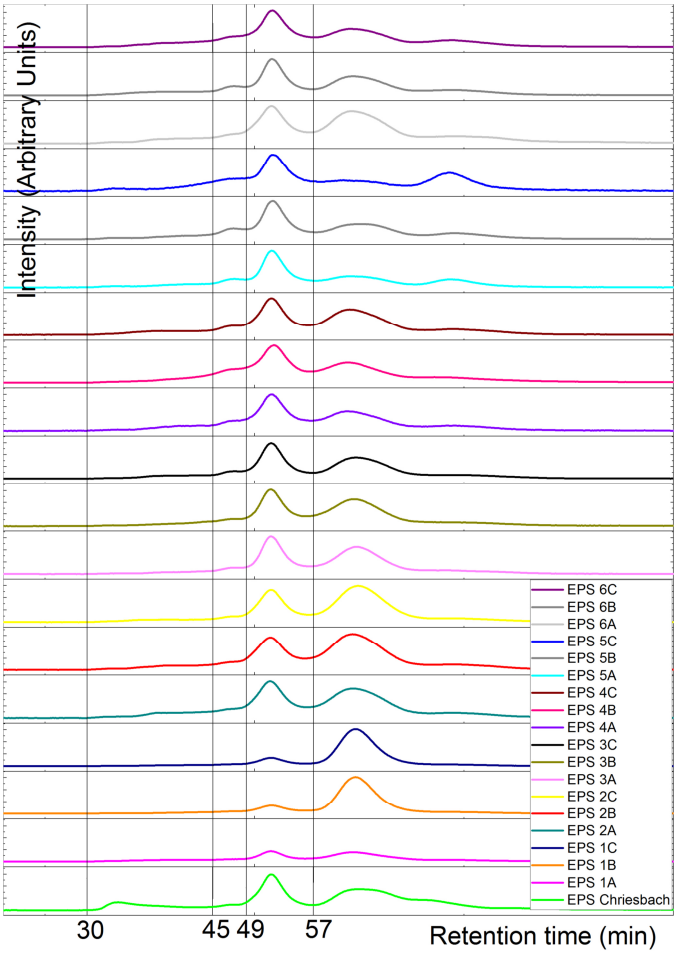
Table S2: Physico-chemical characteristics of the sampling sites along Mönchaltorfer Aa. Silver (Ag107, Ag109) was below detection limit.

	T °C	pH	Conductivity S/m	Flow m/s	Chloride mg/L	Nitrate mg N/L	Sulfate mg/L	Na mg/L	Mg mg/L	Ca mg/L	K mg/L	ortho - Phosphate µg/L	dissolved Phosphate µg/L	total Phosphate µg/L
Minimum	8.7	7.35	0.52	0.057	12	0.5	9	8.2	15.3	78.5	2.1	1.6	2.7	13.1
Median	11.05	7.82	0.68	0.15	31	3.95	30.5	16.35	25	91.85	4.25	10.35	15.5	30.55
Maximum	13.7	8	1.21	0.24	164.1	16	40	107.7	28.8	99.8	13.8	231	236	321

	H_4SiO_4 mg/L	DOC mg/L	TOC mg/L	B11 µg/L	Cd111 µg/L	Co59 µg/L	Cr52 µg/L	Cu65 µg/L	Fe56 µg/L	Mn55 µg/L	Ni60 µg/L	Pb208 µg/L	Zn66 µg/L
Minimum	4.9	1.7	1.8	20.2	0.005	0.02	0.12	0.58	3.19	0.74	0.27	0.04	0.24
Median	12	2.8	3.15	54	0.019	0.09	0.195	1.48	9.885	6.125	0.65	0.13	1.645
Maximum	16.4	3.9	4.4	135.5	0.055	0.43	0.32	2.3	39.7	22.4	2.46	0.49	19.2

Figure S1: LC-OCD-OND chromatograms of starch, glucose, glucuronic acid, *N*-acetyl glucosamine and each EPS sample from 12 sampling sites (A, B and C stand for three replicas).





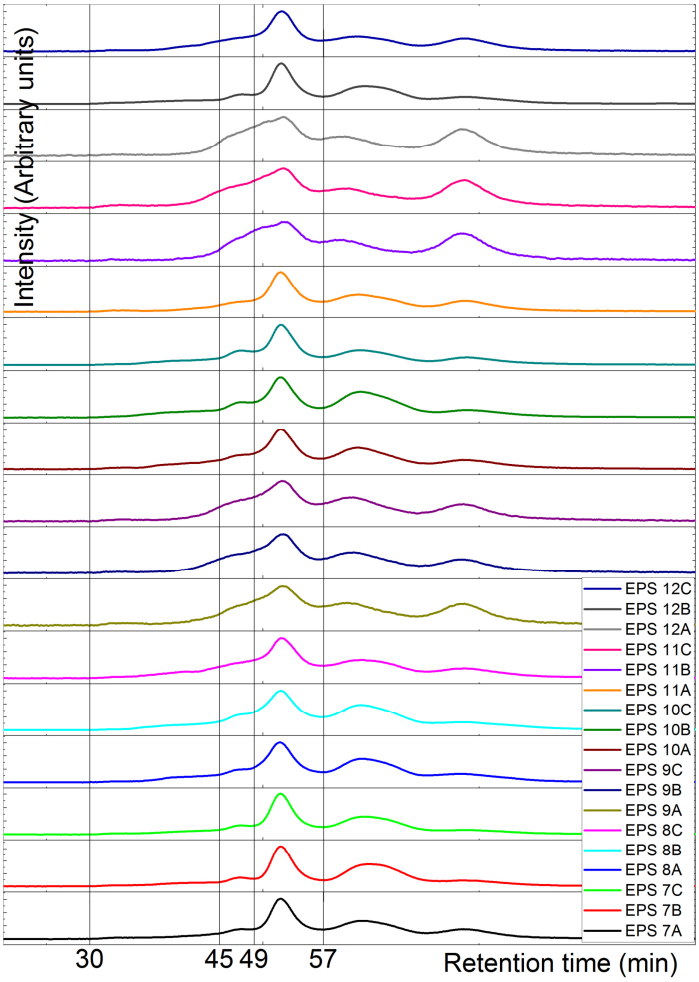

Intensity (Arbitrary units)
30
45 49
57
Retention time (min)
EPS 12C
EPS 12B
EPS 12A
EPS 11C
EPS 11B
EPS 11A
EPS 10C
EPS 10B
EPS 10A
EPS 9C
EPS 9B
EPS 9A
EPS 8C
EPS 8B
EPS 8A
EPS 7C
EPS 7B
EPS 7A

Table S3: Characterization of the EPS extracts by LC-OCD-OND, protein and glucose quantification. BP: biopolymers; BB: building blocks of humic acids; LMWA: low molecular weight acids; NA: neutral and amphiphilic compounds.

EPS extract		DOC	BP	BB	LMWA	NA	Protein	Glucose
		µg DOC/ mg dry weight	%	%	%	%	µg protein/ mg dry weight	µM
Site	**R**							
	A	345.38	11.46	5.57	27.26	55.71	13.96	0
1	B	611.55	5.33	4.50	21.02	69.15	9.54	0
	C	1118.52	6.00	4.14	21.43	68.44	11.81	0
	A	241.91	9.82	6.45	30.80	52.93	34.09	0
2	B	450.74	7.81	5.42	27.98	58.79	23.00	0
	C	156.53	6.07	4.30	28.43	61.20	130.98	0
	A	327.35	5.04	5.23	34.87	54.85	15.10	0
3	B	230.98	6.47	5.74	33.34	54.45	34.78	0
	C	96.042	7.58	6.33	34.74	51.35	25.68	0
	A	119.33	9.96	7.89	35.54	46.61	31.66	1.887
4	B	1055.2	8.55	10.11	36.27	45.06	5.66	0
	C	198.75	8.77	6.63	31.99	52.61	19.83	0
	A	49.88	8.76	7.95	39.36	43.92	29.55	0
5	B	86.12	8.47	8.68	36.83	46.02	22.89	151.452
	C	134.08	10.89	9.39	32.86	46.86	42.32	0
	A	455.94	8.36	5.84	28.68	57.12	95.14	0
6	B	351.28	9.79	7.34	33.63	49.24	21.15	0
	C	944.01	9.91	8.25	35.08	46.76	10.85	0
	A	57.15	7.36	7.33	35.09	50.22	18.38	3.238
7	B	54.43	7.28	6.46	33.71	52.55	40.66	34.785
	C	53.36	7.51	7.40	38.10	46.99	18.21	0
	A	511.12	8.84	8.43	33.00	49.72	34.53	0
8	B	800.86	8.65	9.26	32.77	49.32	26.13	0
	C	29.26	11.19	10.30	33.70	44.81	36.72	0
	A	33.48	7.49	10.36	31.61	50.54	22.79	1.572
9	B	38.82	8.26	11.82	33.03	46.89	27.82	91.324
	C	50.32	7.44	11.43	32.10	49.03	17.21	3.358
	A	193.68	10.35	9.49	33.42	46.74	39.02	0
10	B	174.5	10.15	9.29	31.36	49.20	21.05	0
	C	196.04	9.59	10.84	37.51	42.06	22.31	0

	A	62.2	6.47	7.83	36.28	49.43	13.91	8.043
11	B	84.18	5.63	11.50	32.05	50.82	47.55	0
	C	365.66	9.36	11.51	29.71	49.42	26.83	0
	A	104.18	5.88	12.34	31.53	50.25	20.16	1.722
12	B	173.61	8.05	7.16	35.97	48.82	13.00	3.193
	C	75.86	9.32	10.01	35.18	45.49	24.38	0

Figure S2: Relative composition of field EPS extracts as determined by LC-OCD-OND based on **Table 3**.

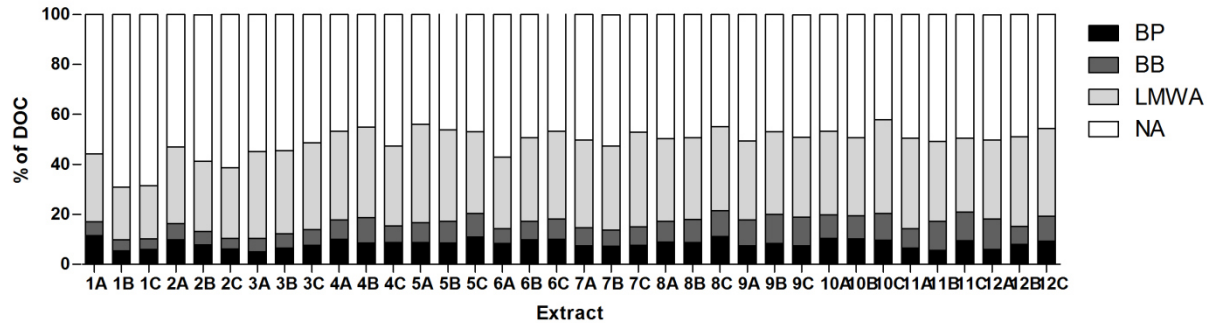


Figure S3: Characterization of nanoparticle dispersions obtained by reacting $AgNO_3$ with field EPS extracts. Wavelengths of the maxima [nm] of the UV-Vis absorption spectra and mean hydrodynamic diameter as determined by NTA.

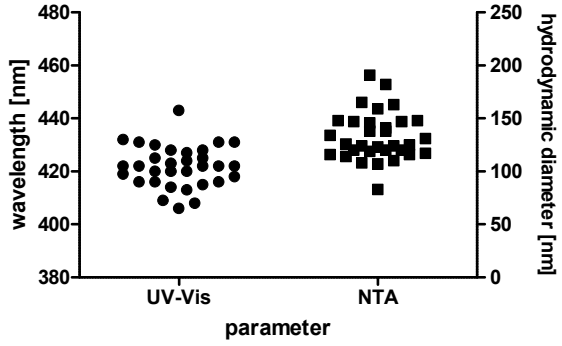


Table S4: Characterization of nanoparticle dispersions obtained by reacting $AgNO_3$ with field EPS extracts. Wavelengths of the maxima [nm] of the UV-vis absorption spectra, mean hydrodynamic diameter [nm] as determined by NTA and DLS and zetapotential [mV] as determined by DLS. Correlation of absorption maxima and mean hydrodynamic diameter was weak (0.55; p=0.001).

EPS extract	UV-Vis	NTA mean	NTA mode	NTA SD	DLS	Zetapotential
2C	424	114	123	44	117	-30.6
3A	422	107	92	51	70	-27.7
3B	420	163	124	60	147	-24.0
3C	416	110	88	40	114	-28.5
4A	406	116	134	42	156	-26.4
4C	428	116	103	42	104	-29.3
5B	420	124	112	43	117	-26.5
5C	414	117	76	48	137	-24.8
6B	408	83	81	27	63	-26.7
6C	409	120	85	50	104	-23.9
7A	427	147	150	66	230	-26.9
7B	425	119	92	60	127	-26.2
7C	422	120	116	53	148	-25.3
8B	425	141	91	63	134	-29.7
9A	418	138	82	94	113	-26.7
9B	420	147	97	74	147	-26.0
9C	432	138	82	88	147	-29.0
11A	431	182	124	77	141	-23.7
11C	431	159	102	67	167	-22.5
12A	415	148	109	66	215	-26.0
12B	428	131	115	53	171	-22.0

The differences in the hydrodynamic diameter values measured by NTA and DLS stem from the differences in the methods. Though both determine the nanoparticle size via analysis of diffusion constants derived from Einstein-Stokes equation (Philippe, Gangloff, Rakcheev, & Schaumann, 2014), NTA tracks the motion of individual particles (Filipe, Hawe, & Jiskoot, 2010), while DLS is based on overall time-dependent scattering intensity fluctuations of the sample (James & Driskell, 2013). Thus, DLS results are strongly influenced by the size dependence of the light scattering, especially prominent for particles smaller than 100 nm (James & Driskell, 2013). To minimize the NTA-based errors caused by the polydispersity of the sample, 3 different areas of each sample were measured for 60 s and the average values were taken.

Figure S4: SERS spectra of nanoparticle controls: citrate (blue), carbonate (red) and EPS on silver films (black). The peaks at 1361 and 1572 cm^{-1} are assigned as COO^- vibrations (see Ref. 37 of the main paper).

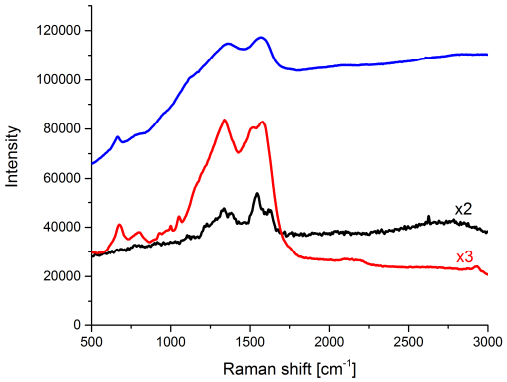


Table S5: Glucose concentration of extracted EPS samples compared to time elapsed until detectable NP formation.

EPS extract	Glucose [pmol/µL]	NP detected (NTA, DLS, UV-Vis) 4 d	8 d	12 d
1A	0			
1B	0			
1C	0			
2A	0			
2B	0			
2C	0			x
3A	0		x	x
3B	0			x
3C	0		x	x
4A	1.887		x	x
4B	0			
4C	0			x
5A	0			
5B	151.452	x	x	x
5C	0			x
6A	0			
6B	0			x
6C	0			x
7A	3.238		x	x
7B	34.785		x	x
7C	0			x
8A	0			
8B	0			x
8C	0			
9A	1.572		x	x
9B	91.324	x	x	x
9C	3.358		x	x
10A	0			
10B	0			
10C	0			
11A	8.043		x	x
11B	0			
11C	0			x
12A	1.722		x	x
12B	3.193		x	x
12C	0			

Table S6: Concentration of selected ions in extracted EPS samples [mg/L]. Chemical species distribution was modelled with VMinteq, which is a chemical equilibrium model software used for the calculation of metal speciation, solubility equilibria, sorption etc. for natural waters. This analysis suggests that in all cases Ag was 100 % dissolved with up to 7 % being present as AgCl (aq). EPS extract 4B was not analysed due to low sample volume.

EPS extract	Cl^-	NO_3^-	SO_4^{2-}	Na^+	Mg^{2+}	Ca^{2+}	K^+
1A	3.7	14.5	<5	70.4	<2.5	8.6	6.0
1B	3.8	16.1	<5	69.3	<2.5	7.4	3.4
1C	4.4	16.2	<5	71.8	<2.5	7.8	4.6
2A	10.0	15.5	<5	55.0	<2.5	11.3	4.4
2B	2.1	12.1	<5	40.8	<2.5	6.8	3.7
2C	6.3	18.3	<5	69.3	<2.5	15.2	11.2
3A	3.1	19.7	<5	80.0	2.7	22.4	4.8
3B	1.5	17.2	<5	73.5	<2.5	11.5	2.7
3C	2.7	18.7	<5	81.0	<2.5	17.1	7.8
4A	3.0	17.9	<5	82.0	<2.5	12.9	6.6
4B	na	na	na	na	na	na	na
4C	1.9	16.5	<5	65.5	<2.5	8.4	3.1
5A	3.8	20.6	<5	95.3	4.4	20.7	4.9
5B	2.2	17.7	<5	75.0	<2.5	12.6	4.0
5C	5.0	14.0	<5	56.0	<2.5	15.0	4.5
6A	10.8	20.7	<5	98.5	2.7	14.5	9.5
6B	6.3	19.7	<5	82.6	<2.5	11.7	10.0
6C	3.8	<0.25	<5	22.8	<2.5	6.8	4.0
7A	2.0	11.3	<5	42.3	<2.5	5.0	1.6
7B	1.6	16.4	<5	66.9	3.3	13.7	3.8
7C	1.4	17.0	<5	71.8	3.3	13.8	4.1
8A	3.8	21.3	<5	80.0	<2.5	8.6	5.9
8B	3.2	16.8	<5	72.8	<2.5	6.7	5.8
8C	5.2	18.4	<5	85.0	<2.5	10.2	2.9
9A	5.0	11.0	<5	36.0	<2.5	<5	<1
9B	0.6	8.3	<5	28.4	<2.5	<5	<1
9C	0.8	14.2	<5	56.3	<2.5	<5	<1
10A	7.5	17.7	<5	79.4	<2.5	11.2	8.4
10B	5.6	12.5	<5	61.5	<2.5	10.6	10.7
10C	2.4	11.8	<5	46.7	<2.5	6.6	5.1
11A	1.9	17.3	<5	82.3	<2.5	13.5	2.5
11B	0.9	13.7	<5	53.8	<2.5	<5	<1
11C	0.7	10.5	<5	40.7	<2.5	<5	1.0
12A	1.2	4.6	<5	21.9	<2.5	<5	<1
12B	1.0	3.5	<5	17.0	<2.5	<5	2.0
12C	0.8	7.1	<5	39.5	<2.5	5.4	1.5

Figure S5: Characterization of EPS-stabilized AgNPs. A: EDX analysis, showing presence of Ag L edges, C K edge and O K edge in the sample. Cu K and L edges come from the TEM sample holder. B: Diffraction pattern, showing presence of (111) and (002) planes, characteristic of non-oxidized Ag surface. C-E: TEM images of the formed Ag NPs embedded in the organic matrix of EPS.

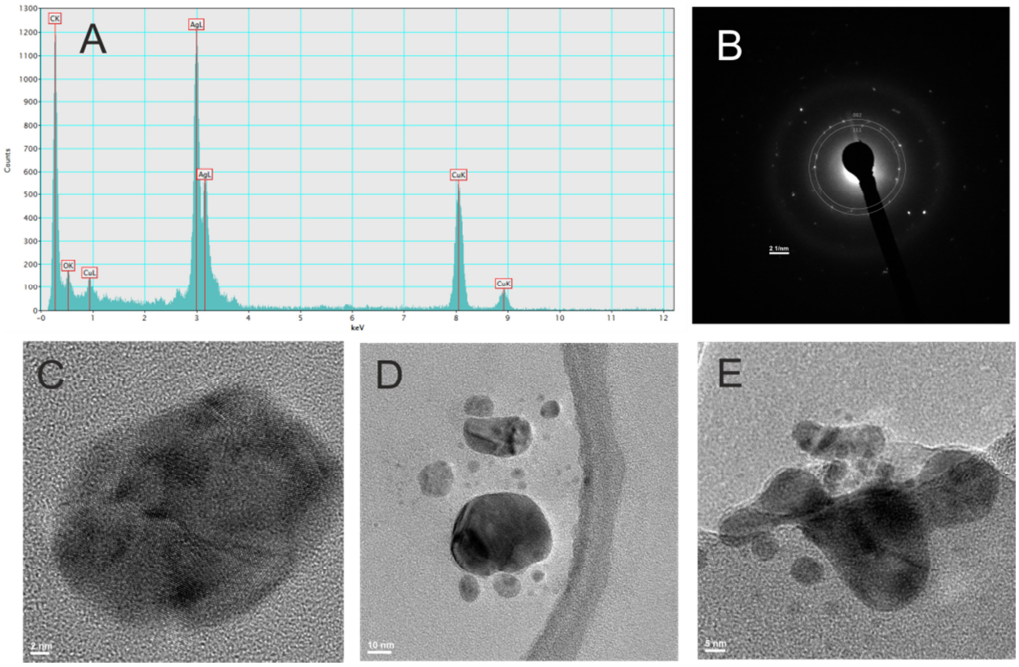
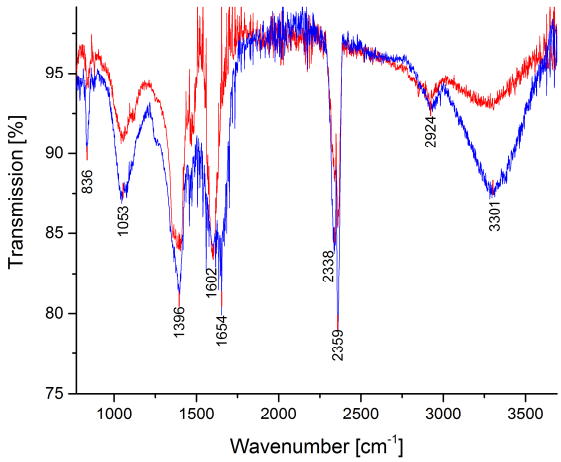


Figure S6: Left: ATR-FTIR spectra of pristine EPS (blue) and EPS-stabilized AgNPs (red) measured in solid state. Right: FTIR peak assignment (Selvakumar, Aravindh, Ashok, & Balachandran, 2013).



Wavenumber [cm^{-1}]	Assignment
1053	C-O-C stretching
1396	C-O stretching in COO
1602	C=O stretching
1654	C=O stretching
2338	O-H
2359	O-H
2924	C-H stretching
3301	O-H stretching