Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supporting information for

Quantitative measurement of nanoparticle size and number concentration from liquid suspensions by atomic force microscopy

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Synthesis and characterisation of Citrate and PVP coated gold nanoparticles

Materials: tetrachloroauric acid ($HAuCl_4.3H_2O$), poly(*N*-vinyl-2-pyrrolidone) with the average molecular weight of 10 000 g/mol (PVP10), trisodium citrate dihydrate $HOC(COONa)(CH_2COONa)_2 \cdot 2H_2O$ and NaOH were purchased from Sigma-Aldrich. These chemicals were analytical grade, and used without further purification. The water used for all reactions and preparations was ultra high purity with a resistivity of 18.2 MΩcm at 25 ^oC.

Synthesis of PVP-coated AuNPs

A detailed method of the process was given elsewhere[1]. Briefly, an aqueous solution of tetrachloroauric acid was added to a solution of polyvinylpyrrolidone (PVP) with vigorous stirring at room temperature. Then aliquot (100 ml) of 0.1M NaOH solution was added to initiate the reduction of the gold ions which then was capped and stabilised by PVP. The synthesized NPs were used without any further purification, cleaning or filtration.

Synthesis of citrate-coated AuNPs

AuNPs were prepared by citrate reduction of $HAuCl_4.3H_2O$ according to the procedure by Turkevich[2] later modified by Frens[3] and others[4]. A 100 ml stock solution of 25 mM Hydrogen tetrachloroaurate was prepared using ultra high purity (UHP) water, of which one ml was further diluted in 99 ml of UHP water. A second stock solution of trisodium citrate was prepared. The diluted solution of $HAuCl_4$ was heated to boiling point while stirring vigorously and then 4 ml of the trisodium citrate stock solution was added quickly. The solutions were kept at the boiling point for 15 minutes to ensure completion of the reaction and were finally allowed to cool to room temperature. The synthesized NPs were used without any further purification, cleaning or filtration.

Centrifugation equations

$$t = \frac{18\mu ln\left({}^{R_2}/_{R_1}\right)}{d^2(\rho_p - \rho_w)w^2} \qquad \text{Eq.S1}$$

Where

t is the time required for the particles to reach the AFM substrate (seconds)

μ is the viscosity of the water (Pa.S)

R_2 is the distance of the AFM substrate from the axis of the ultracentrifuge (cm)

R_1 is the distance of the upper liquid in the centrifuge tube from the axis of the ultracentrifuge (cm)

d is the nanoparticle diameter (cm)

ρ_p is the density of the AuNPs (g cm^{-3})

ρ_w is the density of the water (g cm^{-3})

w is the angular velocity of (rad S^{-1}) and can be calculated according to Eq.S2

$$w = \frac{2\pi RPM}{60} \qquad \text{Eq.S2}$$

Where

RPM is the centrifuge speed in revolution per minutes

Table S1: Physicochemical properties of citrate and PVP coated AuNPs measured by different analytical techniques.

Coting of AuNPs	Z-dh (nm) (polydispersity index) DLS	Number average Hydrodynamic diameter (nm) NTA	Equivalent circular diameter (nm) TEM	Particle height (nm) AFM	Zeta Potential (mV) DLS	Wavelength of maximum UV-vis absorbance
PVP	20.6 (0.15)	53.25	10±2.8	12.2±2.2	-8.31	525
Citrate	21.4 (0.17)	23	15±3.3	13.3±2.1	-43	519

Z-dh: z-average hydrodynamic diameter
DLS: dynamic light scattering
NTA: nanoparticle tracking analysis
AFM: atomic force microscopy
UV-vis: ultraviolet-visible scpectroscopy
Shape factor represents the circularity of the projected images of the NPs.

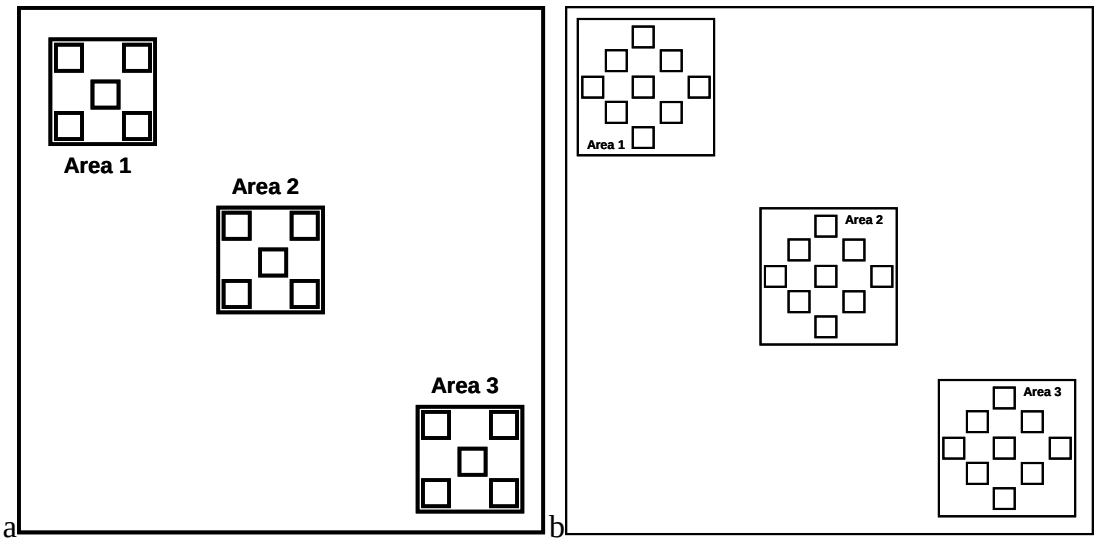

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Figure S1. Areas and images scanned by AFM for each sample. Three areas at different locations on the AFM substrate and (a) 5 and (b) 9 images were collected by area, resulting in collecting 15-29 images for each sample.

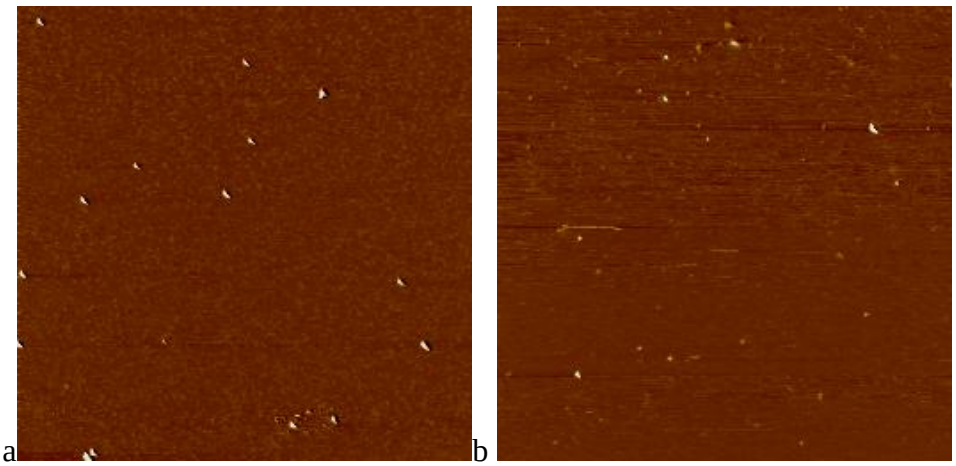

Figure S2. Typical AFM micrographs (5 μm x 5 μm) of Cit-AuNPs in ultrahigh purity water (UHPW) prepared on freshly cleaved bared mica substrate at different concentrations (a) 101.6 ppb Au and (b) 40.64 ppb Au.

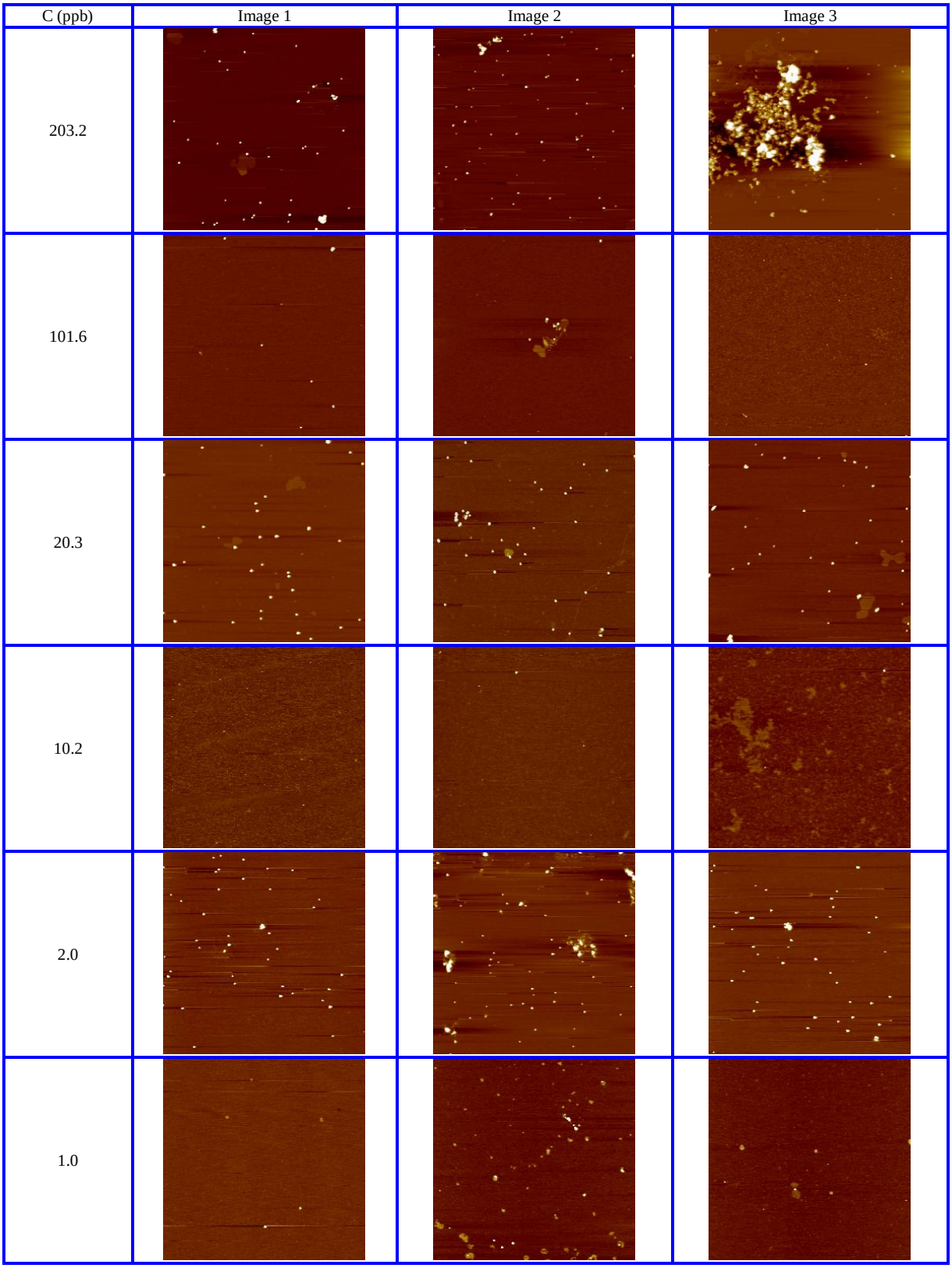

Figure S3. Typical AFM micrographs (5 µm x 5 µm) of Cit-AuNPs suspended in 100 µM $CaCl_2$ and ultracentrifuged on freshly cleaved bared mica substrate at different concentrations of AuNPs (1-203.2 ppb) expressed as ppb Au.

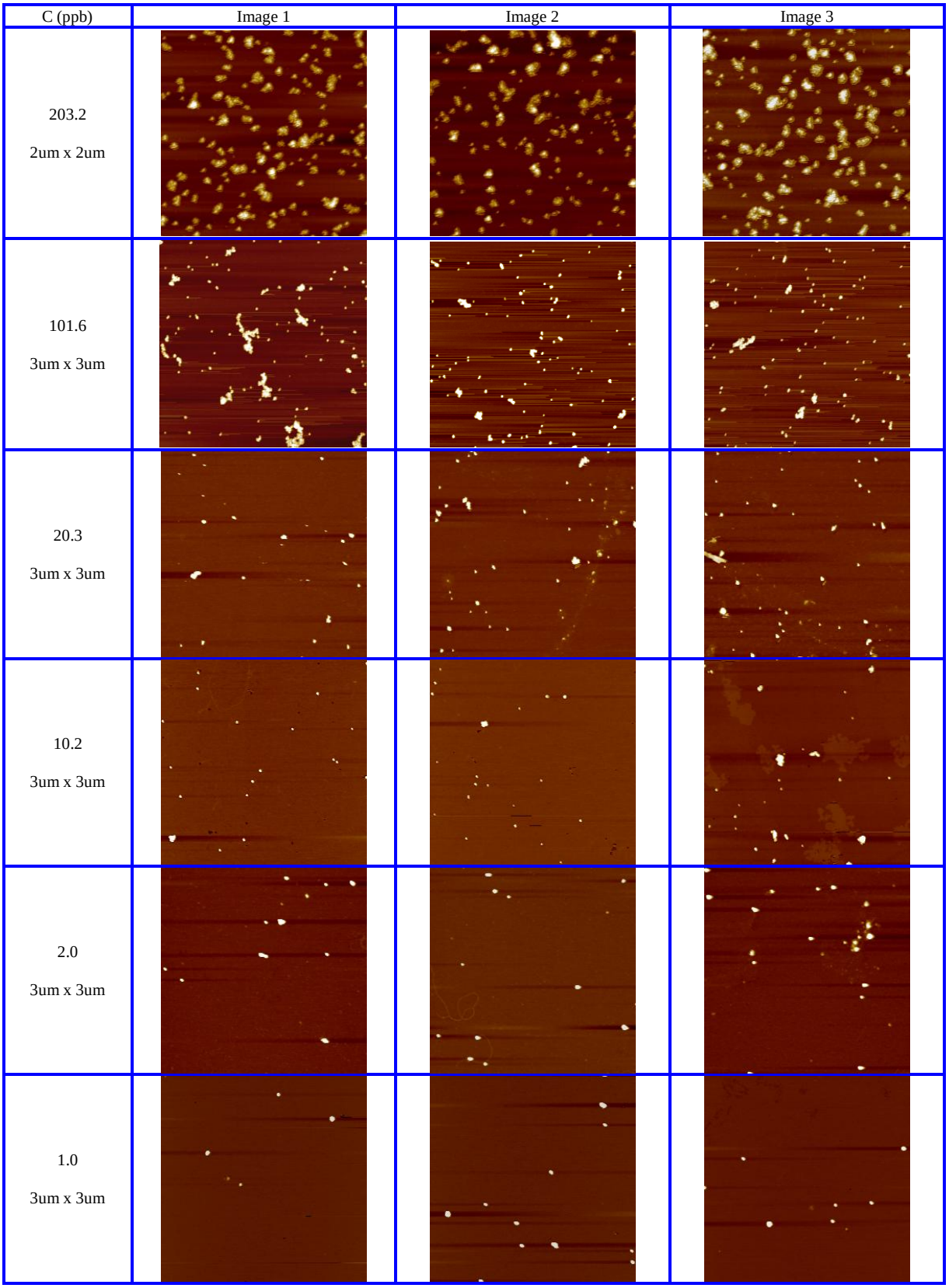

Figure S4. Typical AFM micrographs of Cit-AuNPs suspended in 200 μM $CaCl_2$ and ultracentrifuged on freshly cleaved bared mica substrate at different concentrations of AuNPs (1-203.2 ppb) expressed as ppb Au.

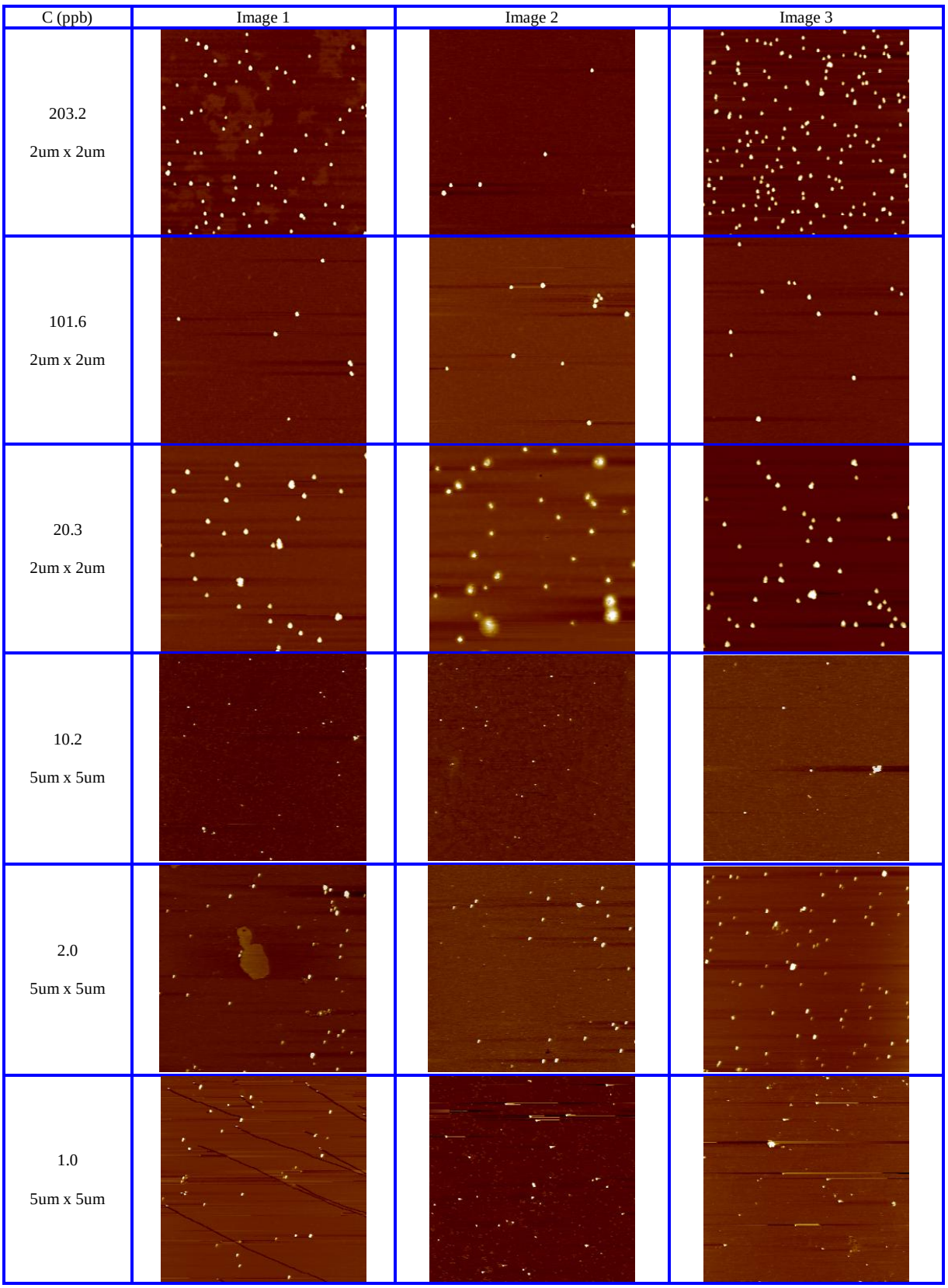

Figure S5. Typical AFM micrographs of Cit-AuNPs suspended in 300 μM $CaCl_2$ and ultracentrifuged on freshly cleaved bared mica substrate at different concentrations of AuNPs (1-203.2 ppb) expressed as ppb Au

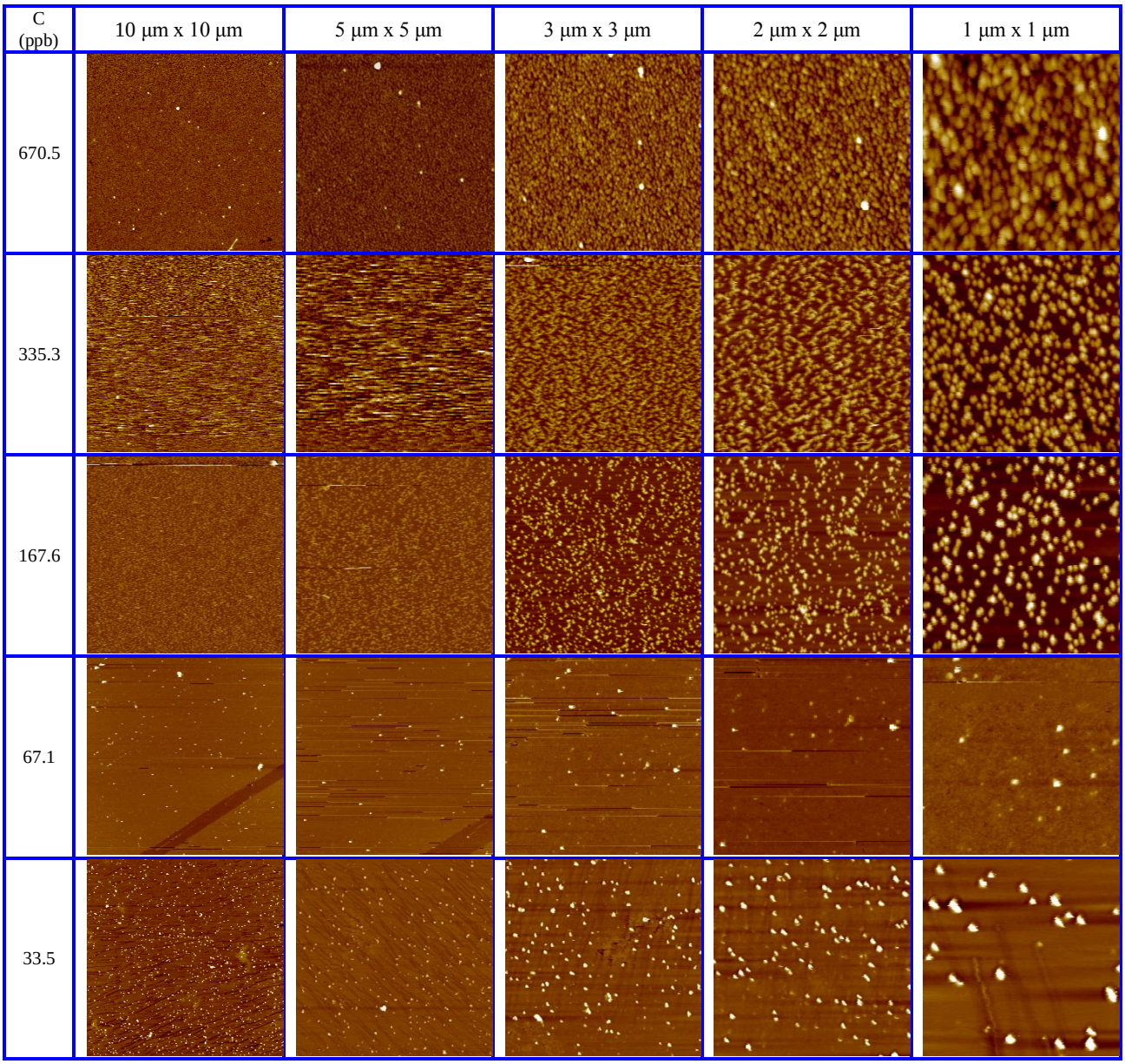

Figure S6. Typical AFM micrographs (5 µm x 5 µm) of PVP-AuNPs suspended in UHPW and ultracentrifuged on freshly cleaved bared mica substrate at different concentrations of AuNPs (33.5-670.5 ppb) expressed as ppb Au. Batch 01

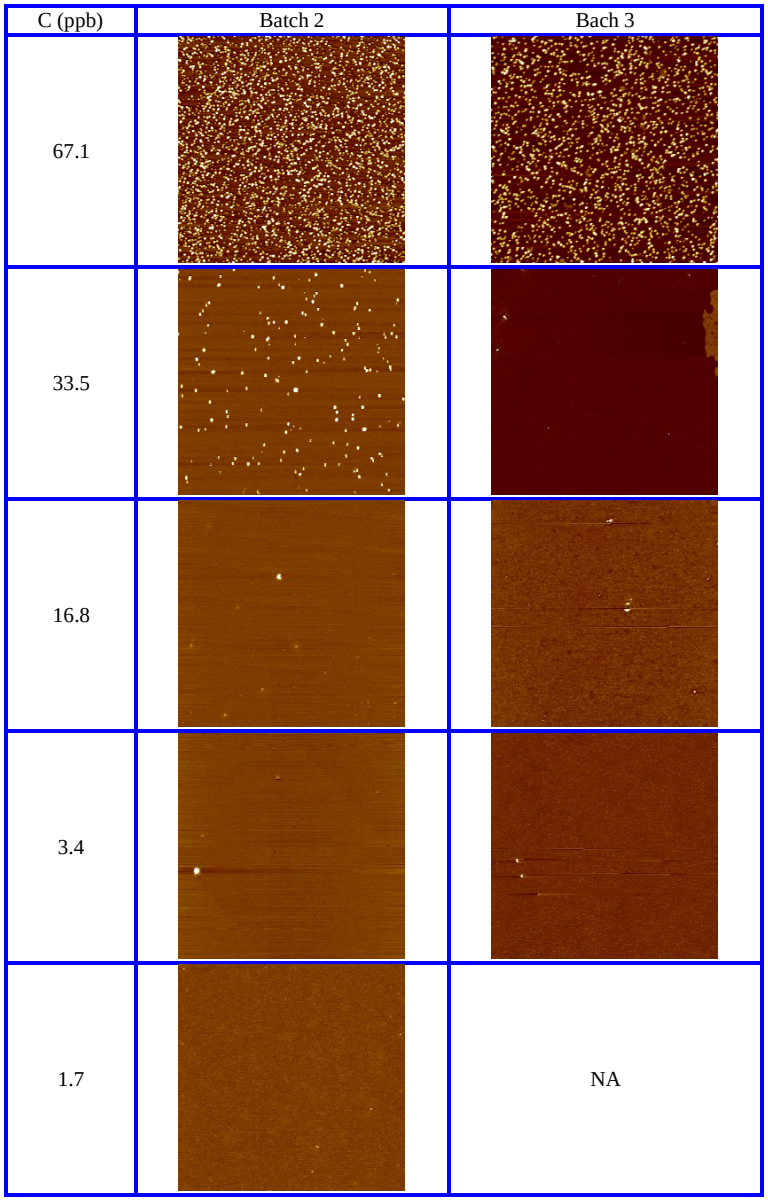

Figure S7. Typical AFM micrographs of PVP-AuNPs suspended in UHPW and ultracentrifuged on freshly cleaved bared mica substrate at different concentrations of AuNPs (1.7-67.1 ppb) expressed as ppb Au. Batch 2 and 3

NA: not analysed

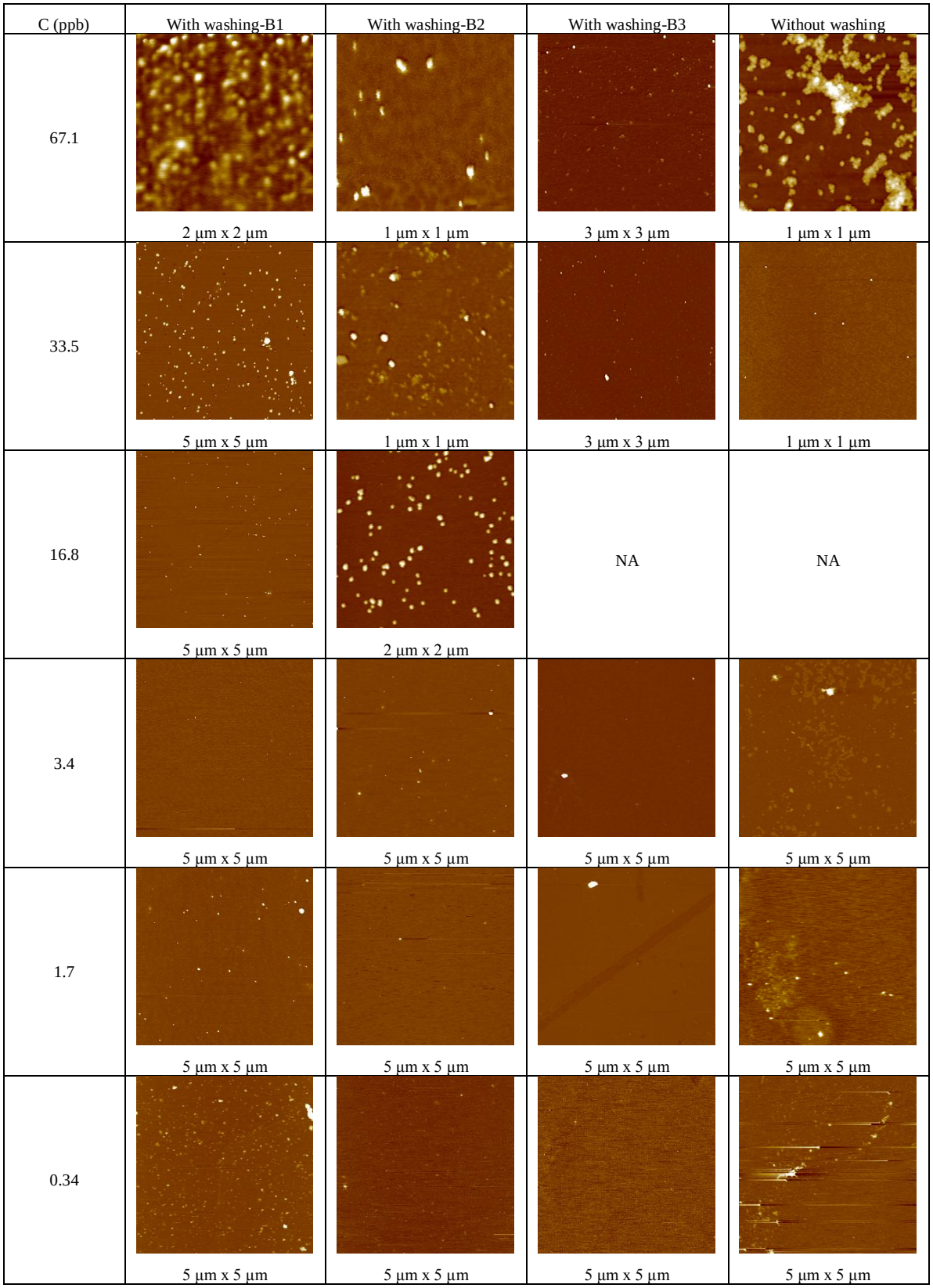

Figure S8. Typical AFM micrographs of PVP-AuNPs suspended in UHPW and ultracentrifuged on poly-l-lysine functionalized mica substrate at different concentrations of AuNPs (1.7-67.1 ppb) expressed as ppb Au. NA: not analysed

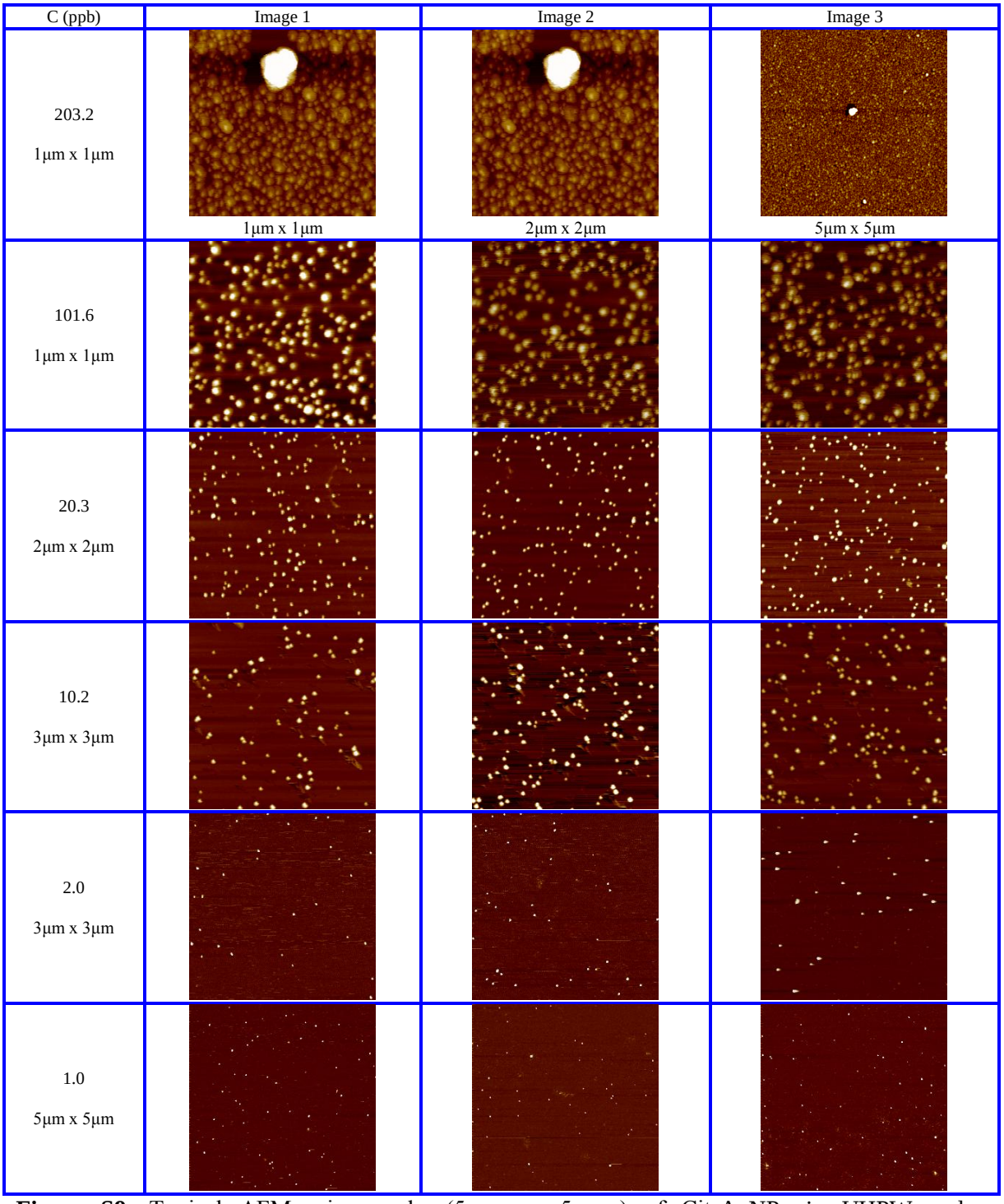

Figure S9. Typical AFM micrographs (5 µm x 5 µm) of Cit-AuNPs in UHPW and ultracentrifuged on poly-l-lysine functionalized mica substrate at different concentrations of AuNPs (1.0-203.2 ppb) expressed as ppb Au.

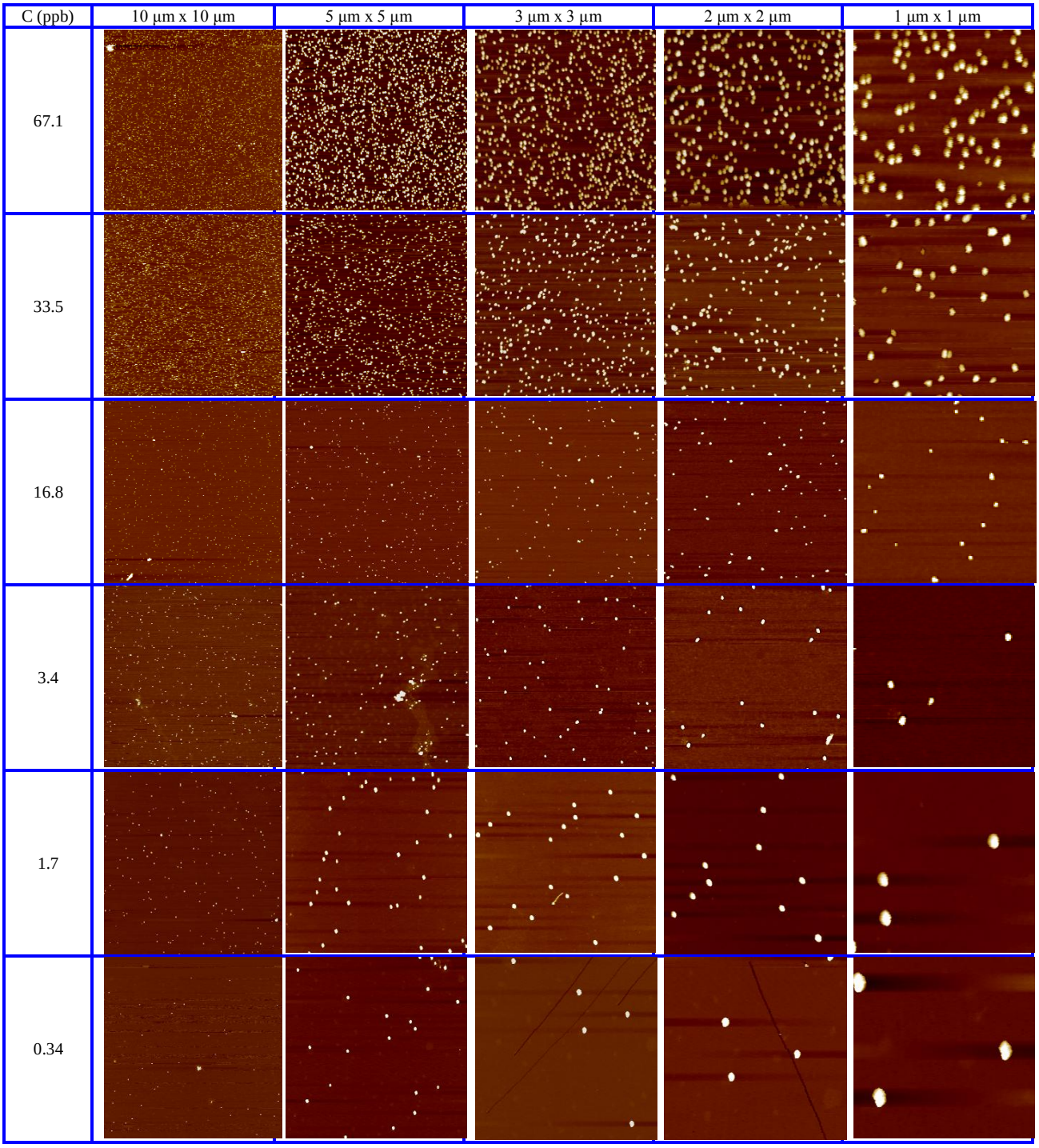

Figure S10. Typical AFM micrographs of PVP-AuNPs suspended in 10 mM $CaCl_2$ and ultracentrifuged bared mica substrate at different concentrations of AuNPs (0.34-67.1 ppb) expressed as ppb Au.

Table S2. Number of NPs counted per μm^2 of AFM substrate for cit-AuNPs

Concentration (ppb Au)	UHPW-B1 CV	UHPW-B2 CV	100 μM $CaCl_2$ CV	200 μM $CaCl_2$ CV	300 μM $CaCl_2$ CV	Poly-l-lysine B1 CV	Poly-l-lysine B2 CV
203.2	NA	NA	2.5±1.0 (0.42)	34.1±2.1 (0.06)	19.9±14.5 (0.73)	OL	OL
101.6	0.4±0.1 (0.22)	1.1±0.5 (0.46)	ND	23.8±3.8 (0.16)	4.4±3.2 (0.73)	203.0±19.7 (0.10)	200.7±20.5 (0.10)
20.3	NC	NA	1.5±0.3 (0.24)	4.2±1.4 (0.34)	10.6±2.8 (0.26)	38.9±2.9 (0.07)	38.3±3.2 (0.08)
10.2	NA	NA	ND	2.6±0.7 (0.27)	0.7±0.2 (0.28)	15.4±1.6 (0.10)	15.2±0.9 (0.06)
2.0	NA	NA	1.7±0.4 (0.24)	1.4±0.4 (0.27)	0.7±0.7 (0.93)	3.8±0.5 (12)	3.2±0.6 (0.18)
1.0	NA	NA	ND	0.9±0.3 (0.34)	0.9±0.2 (0.17)	2.1±0.2 (0.08)	1.6±0.2 (0.12)

The number between the brackets is the coefficient of variation of the number of NP per μm^2 of the substrate
NA: Not analysed
ND: not detected/not sufficient number of particles to be counted
OL: overloading
CV: coefficient of variation

Table S3: Number of NPs counted per μm^2 of AFM substrate for PVP-AuNPs

Concentration (ppb Au)	UHPW -B1 CV	UHPW -B2 CV	UHPW -B3 CV	10 mM $CaCl_2$-B1 CV	10 mM $CaCl_2$-B2 CV
670.5, 335.3 and 167.6	Overloading	NA	NA	NA	NA
67.1	ND	147.4±7.8 (0.05)	68.6±43.5 (0.63)	83.6±11.5 (0.14)	102.5±8.3 (0.08)
33.5	19.4±4.8 (0.25)	7.3±1.8 (0.26)	ND	50.8±4.5 (0.09)	31.1±5.3 (0.17)
16.8	NA	ND	ND	16.4±2.1 (0.13)	15.8±1.4 (0.09)
3.4	NA	ND	NA	4.7±1.7 (0.15)	3.9±0.4 (0.10)
1.7	NA	ND	NA	1.9±0.3 (0.14)	2.1±0.2 (0.11)
0.34	NA	ND	NA	0.6±0.2 (0.33)	0.6±0.1 (0.24)

NA: Not analysed
ND: not detected/not sufficient number of particles to be counted
CV: coefficient of variation

Table S4: Number concentration (particle.L^{-1}) of cit-AuNPs in diluted samples

Concentration (ppb Au)	UHPW-B1 CV	UHPW-B2 CV	100 µM $CaCl_2$ CV	200 µM $CaCl_2$ CV	300 µM $CaCl_2$ CV	Poly-l-lysine B1 CV	Poly-l-lysine B2 CV
203.2	NA	NA	2.96×10^{10} 0.42	4.08×10^{11} 0.06	2.38×10^{11} 0.73	OL	OL
101.6	5.90×10^{09} 0.22	1.34×10^{10} 0.46	ND	2.84×10^{11} 0.16	5.3×10^{10} 0.73	2.70×10^{12} 0.10	2.67×10^{12} 0.10
20.3	NC	NA	1.75×10^{10} 0.24	5.00×10^{10} 0.34	1.27×10^{11} 0.26	5.10×10^{11} 0.07	5.09×10^{11} 0.08
10.2	NA	NA	ND	3.12×10^{10} 0.27	9.0×10^{09} 0.28	2.05×10^{11} 0.10	2.02×10^{11} 0.06
2.0	NA	NA	1.99×10^{10} 0.24	1.73×10^{10} 0.27	8.79×10^{09} 0.93	5.05×10^{10} 0.12	4.20×10^{10} 0.18
1.0	NA	NA	ND	1.04×10^{10} 0.34	1.10×10^{10} 0.17	2.77×10^{10} 0.10	2.17×10^{10} 0.12

NA: Not analysed

ND: not detected/not sufficient number of particles to be counted

OL: overloading

WA: waiting analysis

Table S5: Number concentration (particle.L^{-1}) of PVP-AuNPs in diluted samples

Concentration (ppb Au)	UHPW -B1 CV	UHPW -B2 CV	UHPW -B3 CV	10 mM $CaCl_2$-B1 CV	10 mM $CaCl_2$-B2 CV
670.5, 335.3 and 167.6	Overloading	NA	NA	NA	NA
67.1	ND	1.76×10^{12} 0.05	8.21×10^{11} 0.63	1.11×10^{12} 0.14	1.36×10^{12} 0.08
33.5	2.50×10^{11} 0.25	9.43×10^{10} 0.26	ND	6.75×10^{11} 0.09	4.13×10^{11} 0.17
16.8	NA	ND	ND	2.17×10^{11} 0.13	2.10×10^{11} 0.09
3.4	NA	ND	NA	6.22×10^{10} 0.15	5.15×10^{10} 0.10
1.7	NA	ND	NA	2.51×10^{10} 0.14	2.74×10^{10} 0.11
0.34	NA	ND	NA	7.58×10^{09} 0.33	7.44×10^{09} 0.24

NA: Not analysed

ND: not detected/not sufficient number of particles to be counted

CV: coefficient of variation

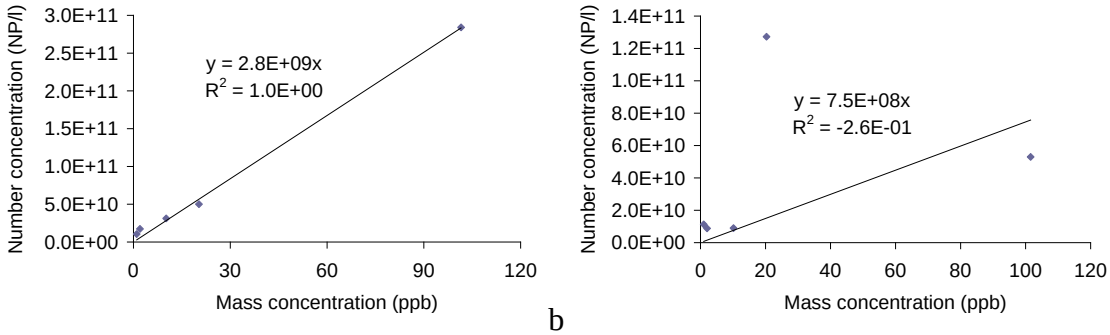

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Figure S11. Correlation between the mass and number concentration of NPs of (a) 200 μm $CaCl_2$ and (b) 300 μm $CaCl_2$

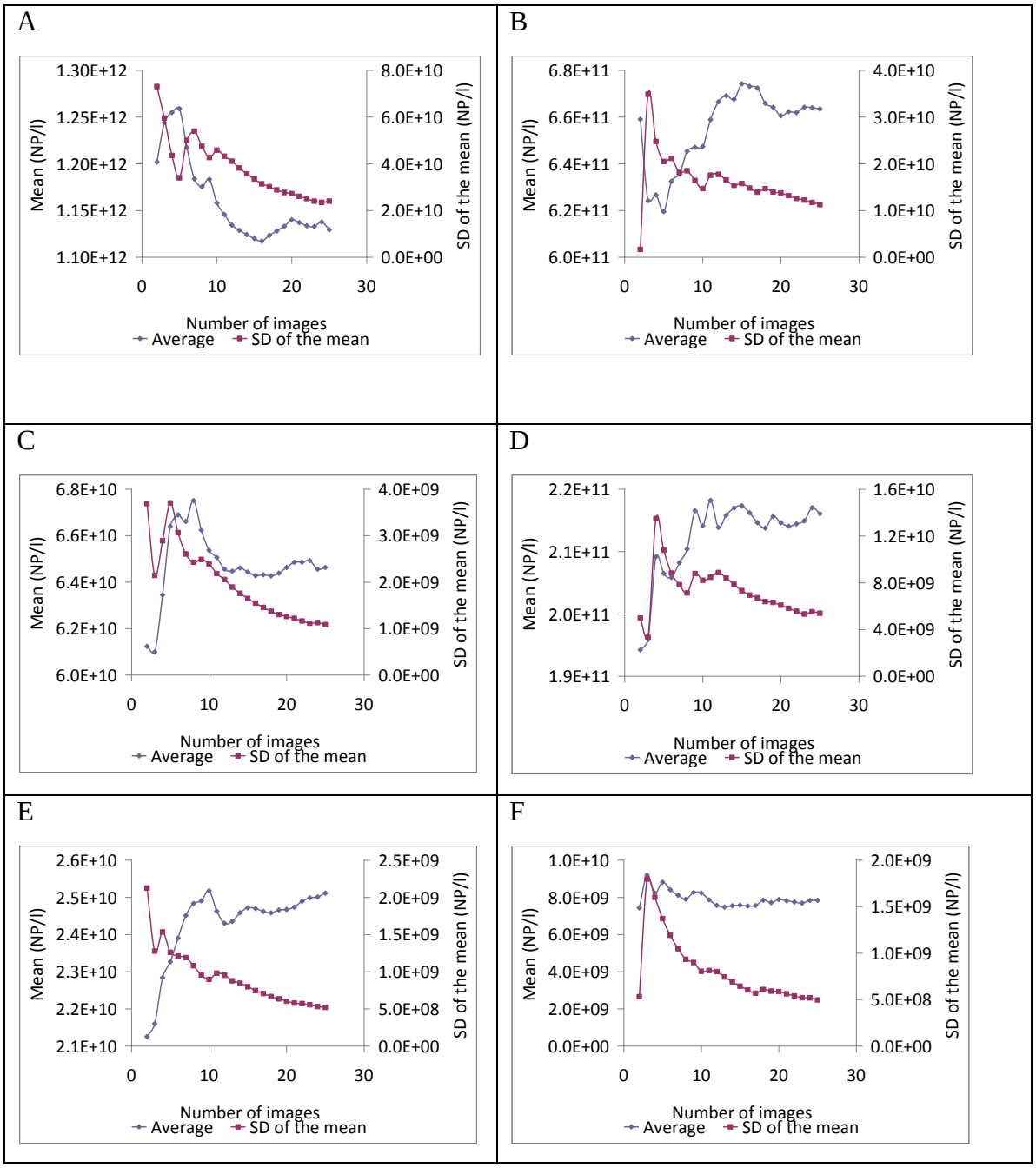
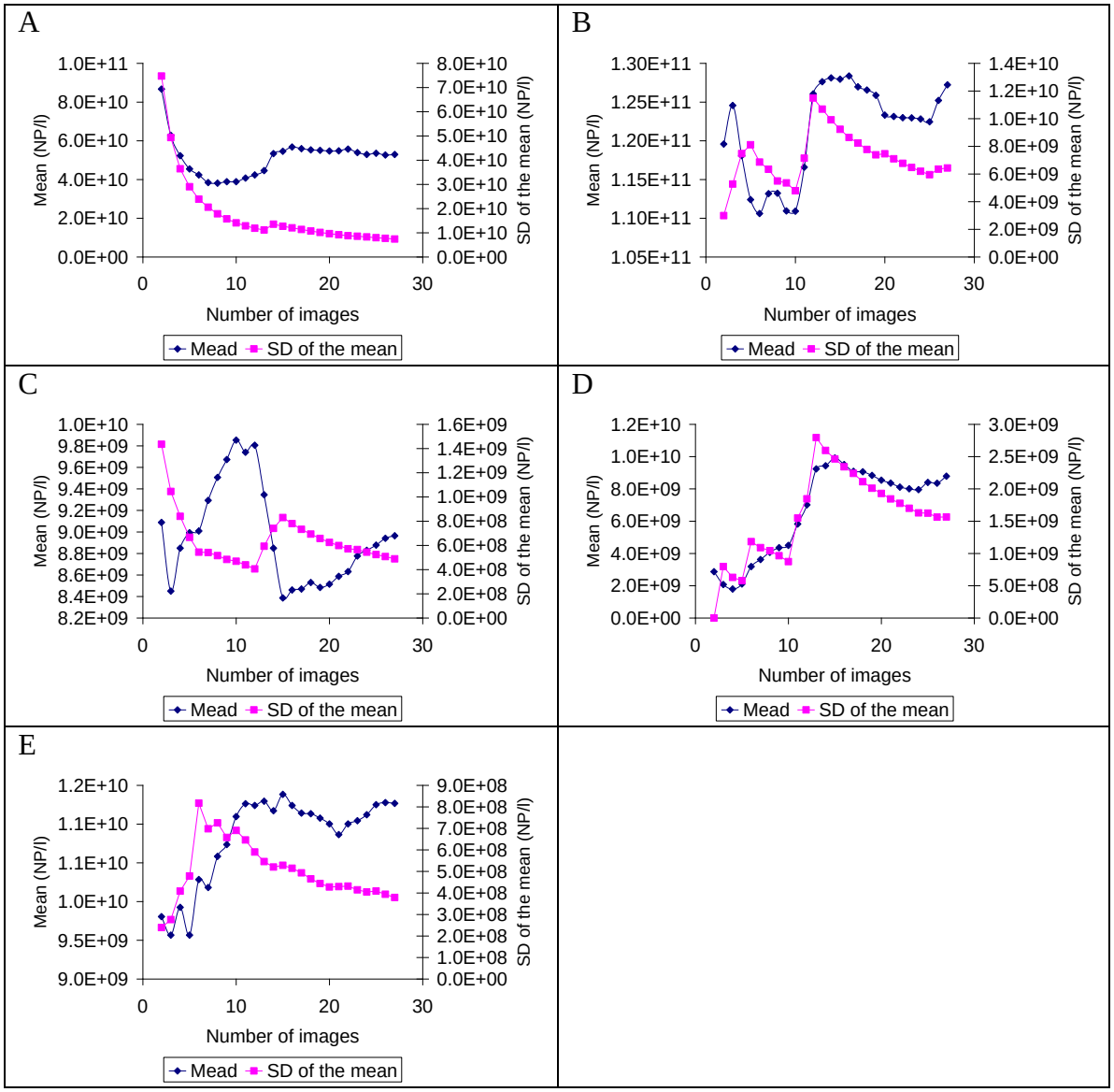


Figure S12. Dependence of the calculated mean number concentration and standard deviation of the mean on the number of images scanned by atomic force microscopy of the PVP-coated AuNPs prepared by adding 10 mM $CaCl_2$ on a bared substrate at different concentrations expressed as ppb Au: (a) 67.1, (b) 33.5, (c) 16.8, (d) 3.4, (e) 1.7 and (f) 0.34.



166 **Figure S13**. Dependence of the calculated mean number concentration and standard
167 deviation of the mean on the number of images scanned by atomic force microscopy of
168 the cit-coated AuNPs prepared by adding 300 μM $CaCl_2$ on a bared mica substrate at
169 different concentrations expressed as ppb Au: (a) 101.6, (b) 20.3, (c) 10.2 (d) 2.0 and (e)
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