Supplementary information

Supplementary Table S1. Metal content of *C. metallidurans* strain AE104 and its Δ*copABCD* mutant[a]

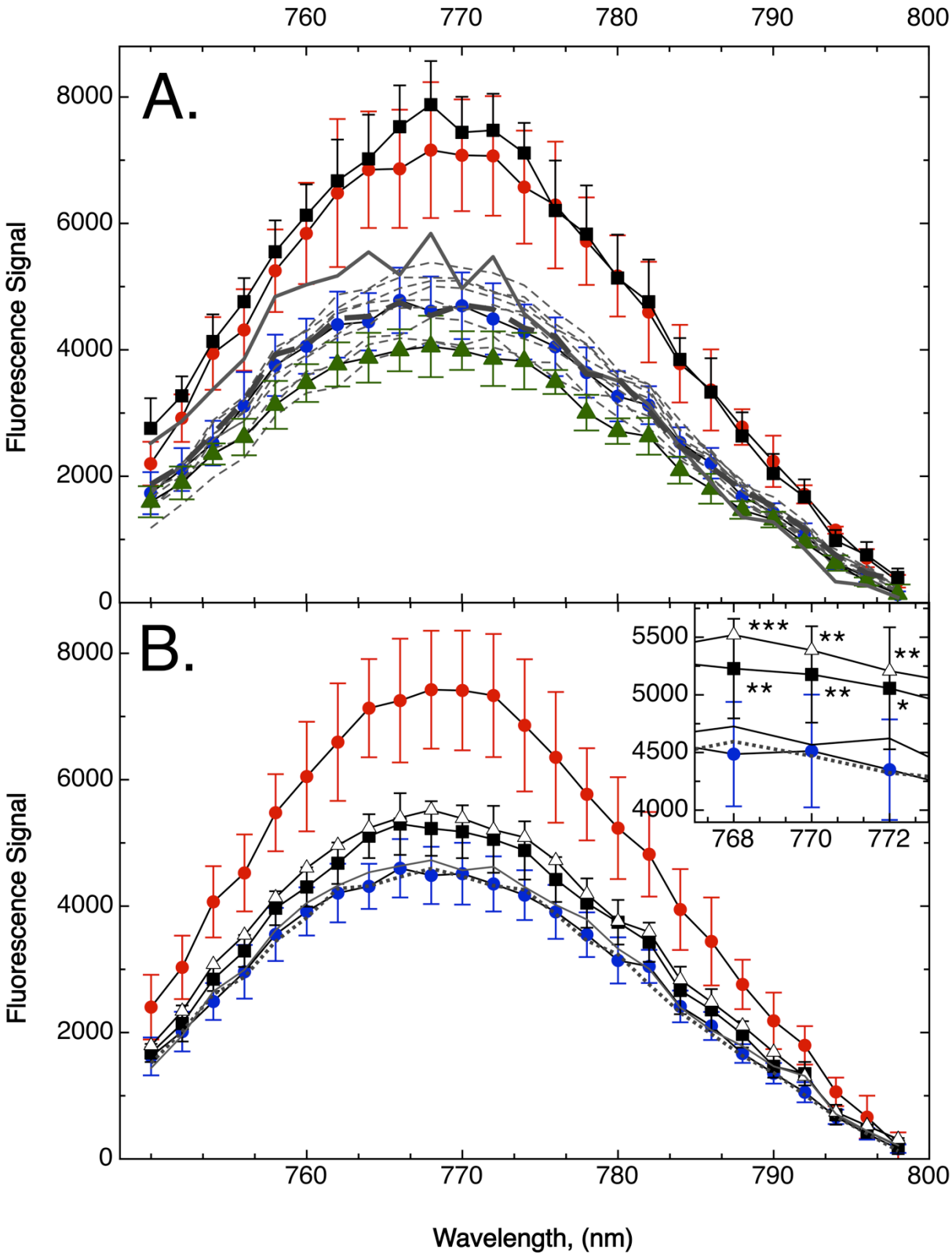
Additions to the pre-culture	Additions to the main culture	Bacterial Strain: AE104	AE104 Δ*cop*	AE104	AE104 Δ*cop*
		Copper, 1000 atoms/cell		Gold, 1000 atoms/cell	
None	None	18±6	12±3	0±0	3±3
None	Au	14±6	3±1	50±7	46±4
	Cu	84±11	112±27	0±0	0±0
	Cu/Au	**140±10**	**283±64**	**176±21**	**104±6**
Cu	Au	46±4	62±9	105±11	119±37
	Cu	66±12	97±32	0±0	2±1
	Cu/Au	73±8	**149±9**	93±8	**201±11**
Au	Au	13±5	11±5	72±10	64±18
	Cu	68±3	82±4	10±3	8±2
	Cu/Au	**144±17**	**194±15**	**192±28**	**116±4**

[a]Cells of the plasmid-free *C. metallidurans* derivative strain AE104 and its *copABCD* mutant were pre-incubated for 24 h in the presence of 2.5 µM Au(III)-chloride, 100 µM Cu(II), or without addition, and diluted 50-fold into the main culture. At a turbidity of 100 Klett units, 2.5 µM Au(III)-chloride, 100 µM Cu(II)-chloride, both metals or none of them were added, incubation was continued with shaking at 30°C until 150 Klett units were reached (about 160 min). The cells were harvested, washed, and the metal content was determined by ICP-MS. Deviations of three independent experiments indicated. The data for Δ*cop* were acquired together with those for AE104 and CH34, which both have been published [1]; the AE104 data are given here for reference. Bold-faced numbers give significant differences between the metal content of cells cultivated in the Cu/Au mixture compared to those grown in presence of one metal only.

Supplementary Table S2. Bacterial strains, plasmids and primers used in this study.

Bacterial strains	**Characterization**	**Reference**
Cupriavidus metallidurans		
AE104	Plasmid-free	[2]
DN686	AE104 Δ*copABCD*	[3]
Escherichia coli		
W3110	Wildtype	[4]
S17/1	Conjugator strain	[5]
BL21	pLysS	Stratagene Europe Amsterdam, The Netherlands
Rosetta	without pLysSRARE	Stratagene Europe Amsterdam, The Netherlands
ECB145	XL1 pECD1569	This study
Plasmids		
pG-Tf2		TAKARA BIO INC., Shiga, Japan
pECD1569	pASK-IBA3plus::$copA_{WM}$	This study
Primers used for cloning of *copA* into pASK-IBA3plus		
Rmet_5671W-M EcoRI ATG	AAAGAATTCATGGCCAGTTCCGGGGGCGGACAG	
Rmet_5671 XhoI TCA	AAACTCGAGTGCCACCACCACTTCACGGAA	

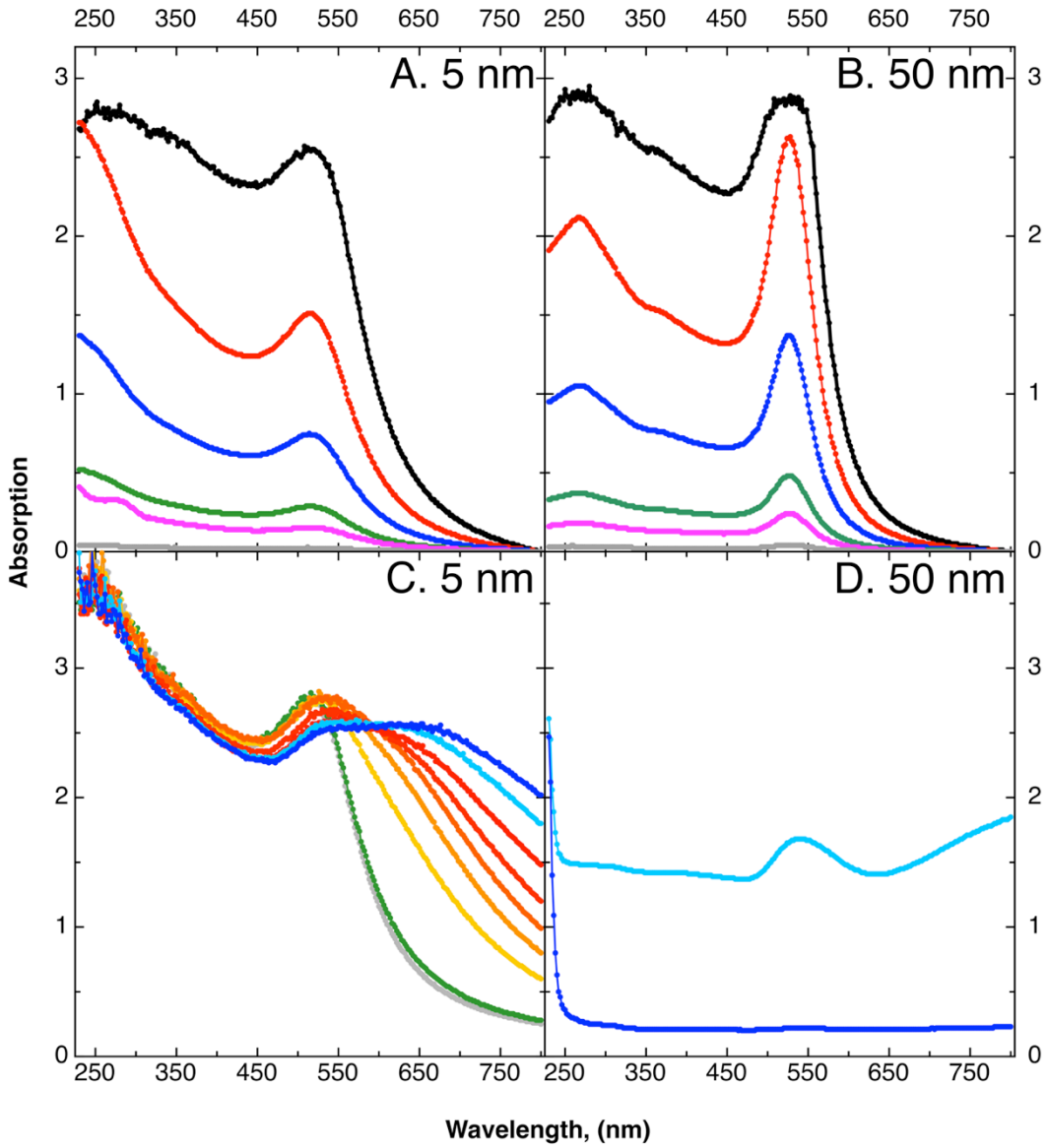
Figures



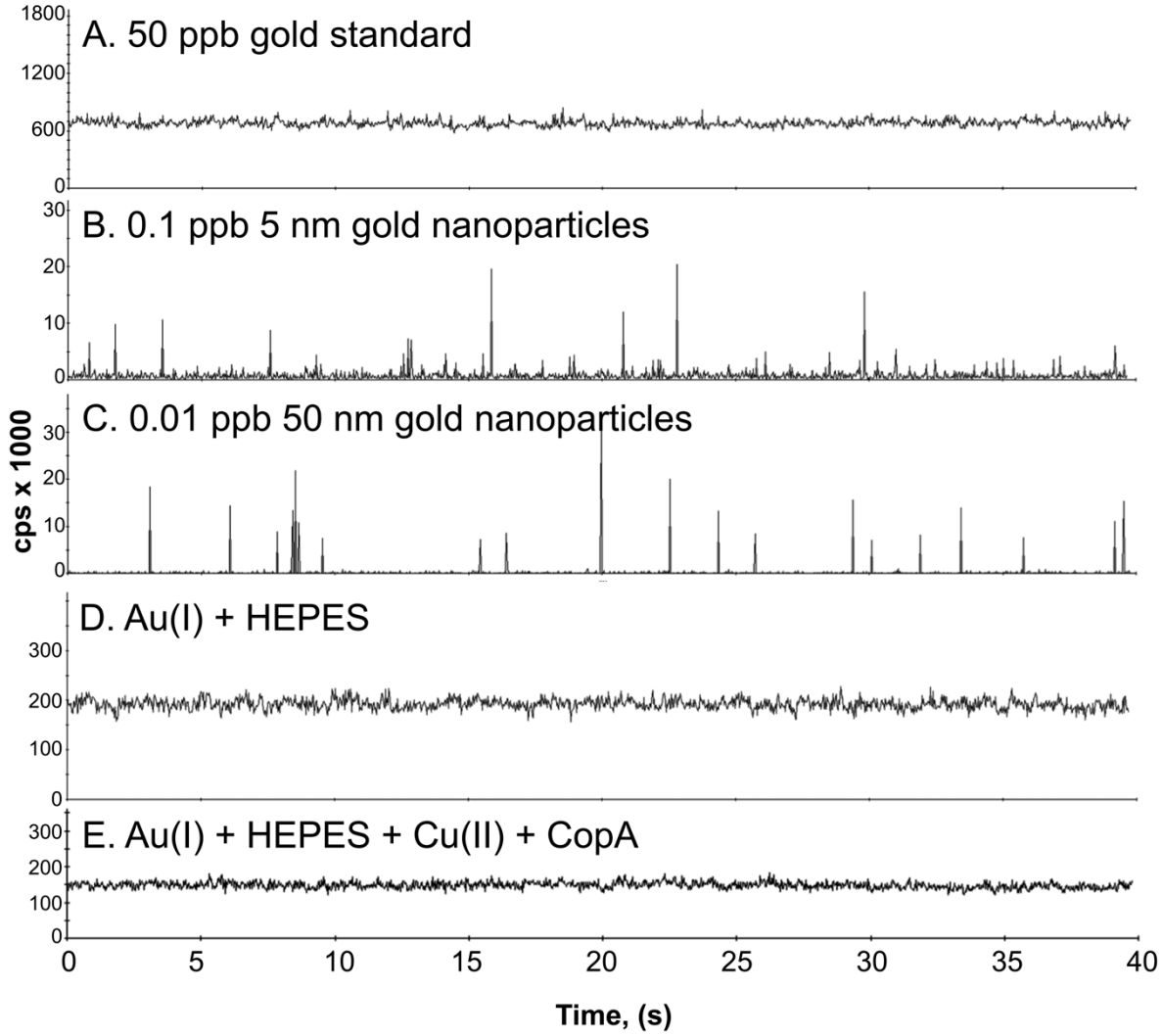
Supplementary Figure 1. CopA is a Cu-dependent Au(I) oxidase. Panel A. Oxygen Nanoprobe particles (0.1 g/L) that are quenched by molecular oxygen [6] were incubated for 10 min aerobically (blue circles, ●, n=7), anaerobically (red circles, ● n=3), with CopA,

$Au(III)Cl_4^-$ plus $Cu(II)Cl_2$ (green triangles, ▲, n=5) or CopA, Au(I) plus $Cu(II)Cl_2$ (black squares, ■, n=5), deviation bars shown, for the black squares only the upper part. The thick grey line is the CopA/Au(I)/$Cu(II)Cl_2$ experiment performed with heat-inactivated CopA, the thick dashed grey line closely following the aerobic buffer control a mixture of CopA, $Cu(II)Cl_2$ and sodium thiosulfate. Concentrations were 50 µM of the respective metal salts and 0.85 nM of CopA. The thin dashed grey lines are additional control experiments with one or two of these three components only, n≥3, deviation bars not shown.

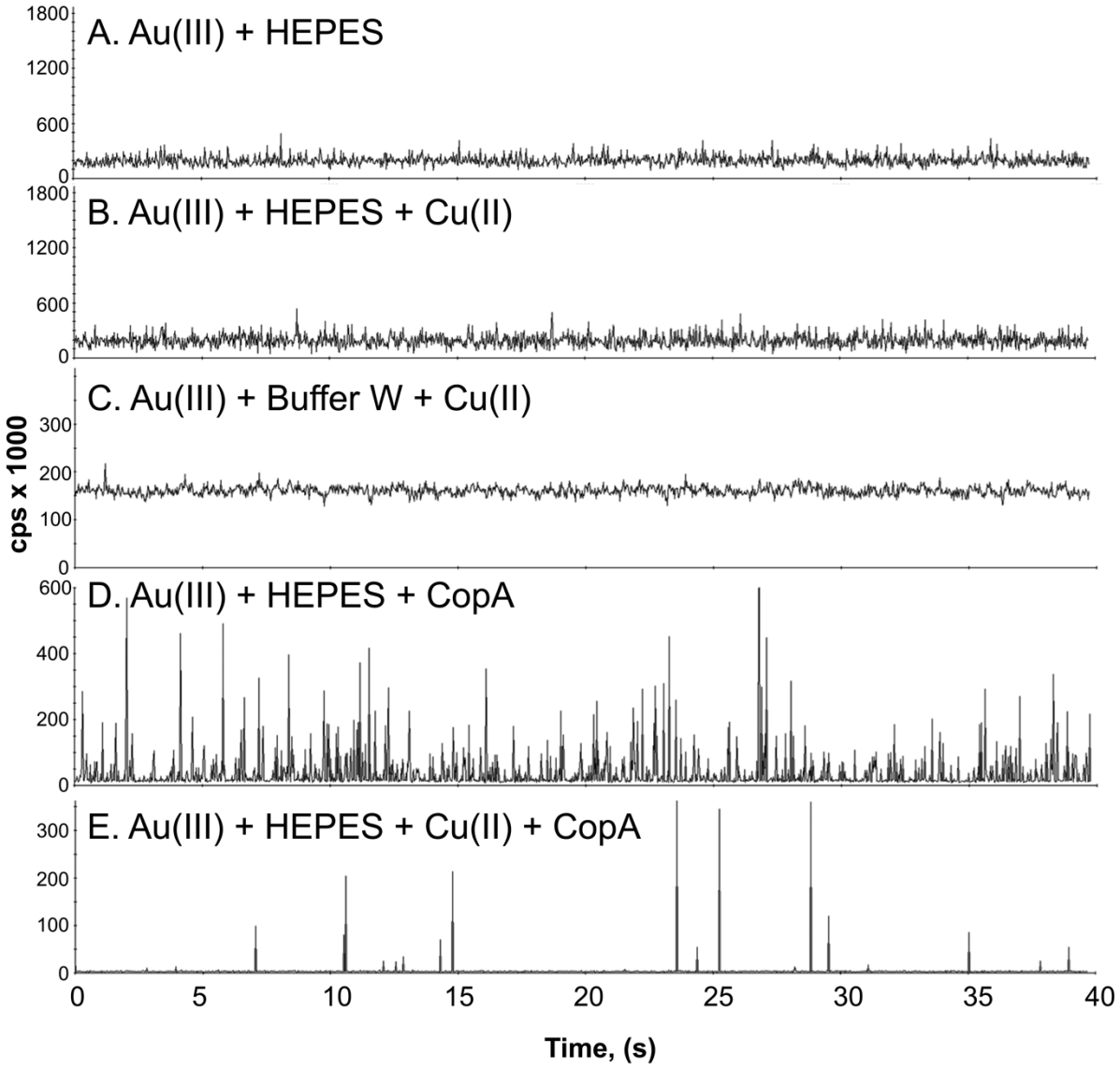
Panel B. CopA was incubated for 10 min on ice with a ten-fold amount of Cu(II), de-salted using a PT10 spin column and used in the subsequent experiment instead of the partially de-metallated CopA shown in Panel A. Oxygen Nanoprobe particles (0.1 g/L) that are quenched by molecular O_2 [6] were incubated for 10 min aerobically (blue circles, ●, n=14), anaerobically (red circles, ●, n=8), with CopA and $Au(I)Cl_4^-$ but without $Cu(II)Cl_2$ (black filled squares, ■, n=6), with CopA, $Au(I)Cl_4^-$ plus $Cu(II)Cl_2$ (open triangles, Δ, n=5), or as further controls with CopA and $Au(III)Cl_4^-$ (dashed line, n=6) or CopA without additions (solid grey line, n=6). Concentrations were 50 µM of the respective metal salts and 0.85 nM of Cu-loaded and de-salted CopA. Deviation bars are shown for the oxic and anoxic buffer controls and CopA plus Au(I). The inlay gives a magnification of the data points around 770 nm indicating significant differences in the oxygen content between the oxic buffer control (blue circles) on the one hand and the CopA/Au(I) or the CoA/Au(I)/Cu(II) experiments on the other hand. Three stars are a highly significant difference according to Students t-test (99.9%), two stars four significant differences (99.5% and 99.0%), one star a statistically meaningful difference (97.5%). Differences between oxic buffer, CopA and CopA/Au(III) were not significant, differences between CopA/Au(I)±Cu(II) (closed squares and open triangles) neither.



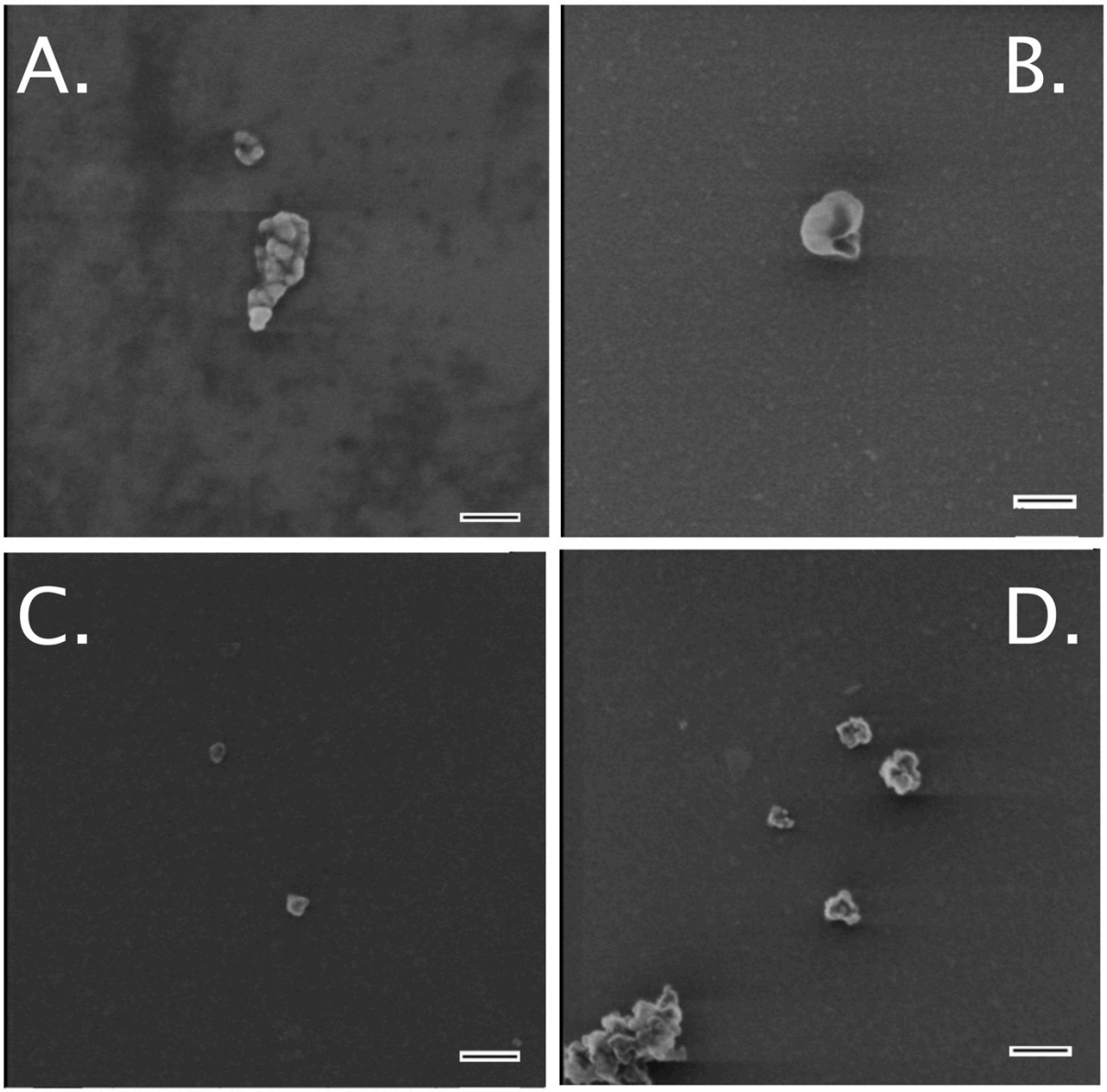
Supplementary Figure S2. Spectrum of commercially available Au nanoparticles and their destabilisation behaviour. Concentration-dependent absorption of commercial BioPure Gold with citrate-stabilized particle surface in water is shown for 5 nm (Panel A) and 50 nm (Panel B) particles. Concentrations used were 0.01 (grey), 0.05 (purple), 0.1 (green), 0.25 (blue), 0.5 (red) and 1 (black) g L^{-1}. Absorption of the 520 nm maximum increased with the concentration of the nanoparticles and was, up to 0.5 g L^{-1}, higher for 50 nm than for 5 nm particles. Additionally, particles were de-stabilised by addition of nitric acid, which resulted a redshift of the color from purple-red to bluish-black, and precipitation of aggregated Au. Particles with a diameter of 5 nm (Panel C, 1 g L-1) were stable for 2 h after addition of 0.38 mM (grey) or 1.1 mM (green) mM nitric acid but aggregated after addition of 2.6 mM (directly after addition, 10, 15, 30, 60 min, from yellow to red) and more quickly after addition of 3.8 mM nitric acid (directly after addition and 15 min, light and dark blue, respectively). 50 nm particles (Panel D, 1 g L^{-1}) aggregated rapidly after addition of 3.8 mM nitric acid (directly after addition light blue, 15 min blue; particles here completely precipitated).



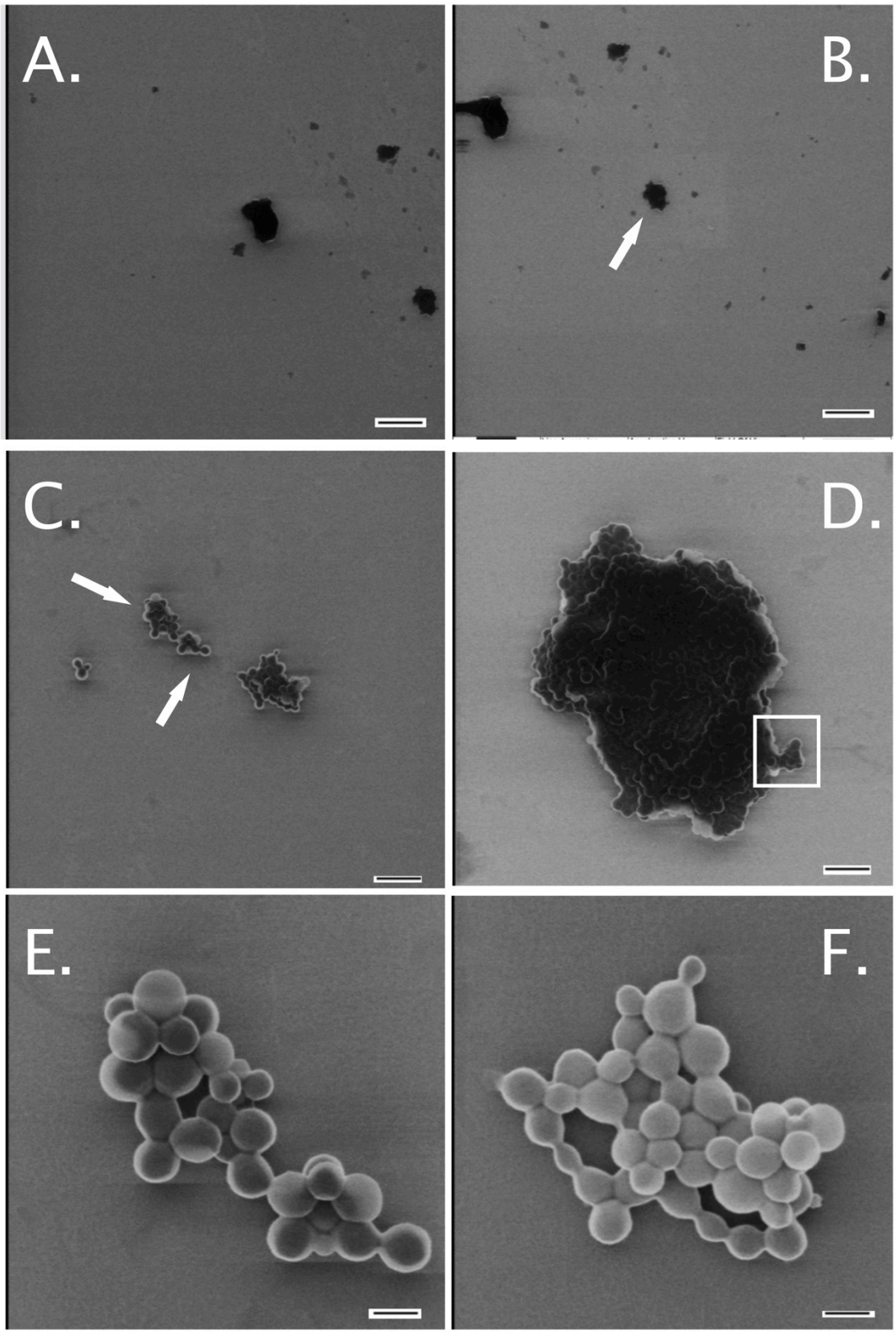
Supplementary Figure S3. Single particle ICP-MS, controls. Single particle ICP-MS analysis of 50 ppb Au197 standard solution used for equilibration (A), 0.1 ppb citrate-stabilised 5 nm Au nanoparticles (B), 0.01 ppb 50 nm nanoparticles (C), 10^{-3}-fold diluted reaction product of Au(I)-thiosulfate in HEPES buffer (D) and the same experiment additionally containing Cu(II) and CopA (E). An elevated baseline indicates dissolved Au species, peaks Au nanoparticles. The samples from the HEPES experiments were diluted 1000-fold into distilled water (analytical grade) and transferred to ICP-MS via auto-sampler, representing about 10 ppb of Au. Dwell time 5 ms.



Supplementary Figure S4. Single particle ICP-MS, experiments. Single particle ICP-MS analysis of the 10^{-3}-fold diluted reaction product of Au(III)-tetrachloride in HEPES (A), additionally containing Cu(II) (B), with buffer W instead of HEPES (C), with CopA instead of Cu(II) (D) and with Cu(II) and CopA (E). An elevated baseline indicates dissolved Au species, peaks Au nanoparticles. The samples from the HEPES experiments were diluted 1000-fold into distilled water (analytical grade) and transferred to ICP-MS via auto-sampler, representing about 10 ppb of Au. Dwell time 5 ms.



Supplementary Figure S5. Helium-ion-microscopic investigation of Au nanoparticles, controls. Particles are shown that are produced in 50 mM HEPES buffer by 50 µM $AuCl_4$ in the absence of 50 µM $CuCl_2$ but presence 0.85 nM CopA (Panel A). The top part of this particle is also shown in Fig. 3B. Moreover, particles are shown that appear in 50 mM HEPES from 50 µM Au(I)-thiosulfate (B, size bar 100 nm) or Au(III)-chloride (Panel C, size bar 1 µm, also shown in Fig. 3A). Panel D shows 50 nm citrate-stabilised commercial Au nanoparticles (D, size bar 100 nm).



Supplementary Figure S6. Helium-ion-microscopic investigation of Au nanoparticles formed by CopA. Particles are shown that are produced in 50 mM HEPES buffer by 50 µM $AuCl_4$ in the presence of 50 µM $CuCl_2$ and 0.85 nM CopA. Panels A and B overview of different grit areas with size bars 5 µm, arrow in B marks particle in D. Panels C and D size bars 500 nm, arrows in C label particles in E and F, square particle shown in Fig. 3D.

References of the supplementary part

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