Supplementary Information

SEEDING to enable sensitive electrochemical detection of biomarkers in undiluted biological samples

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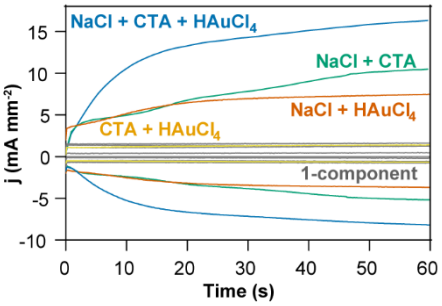
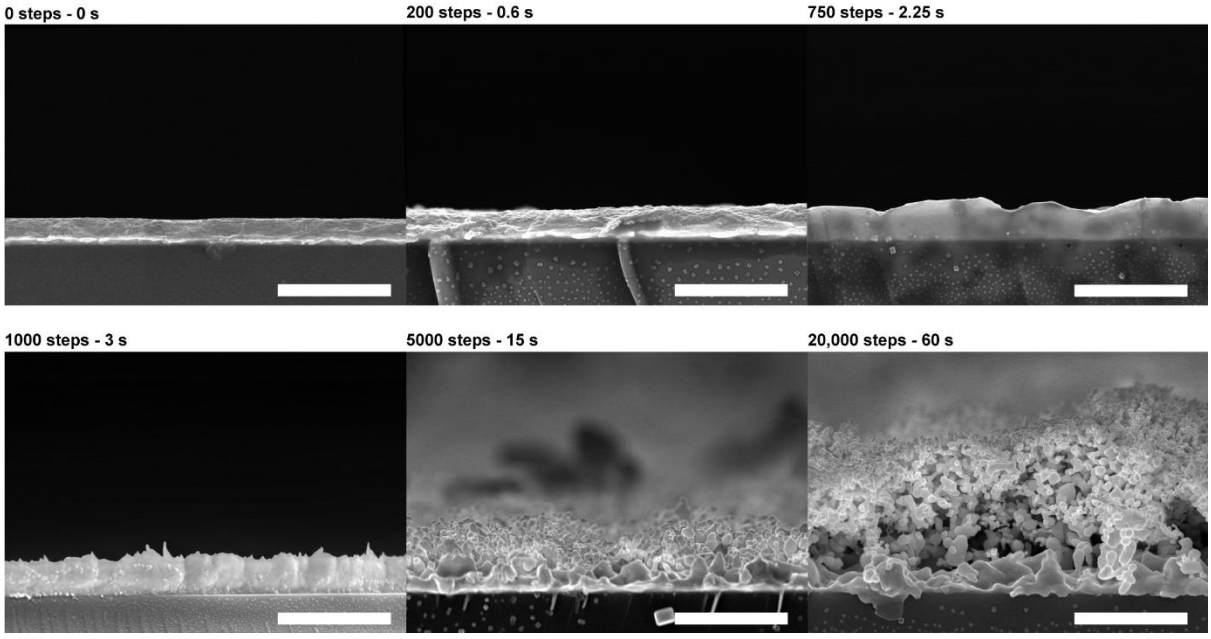
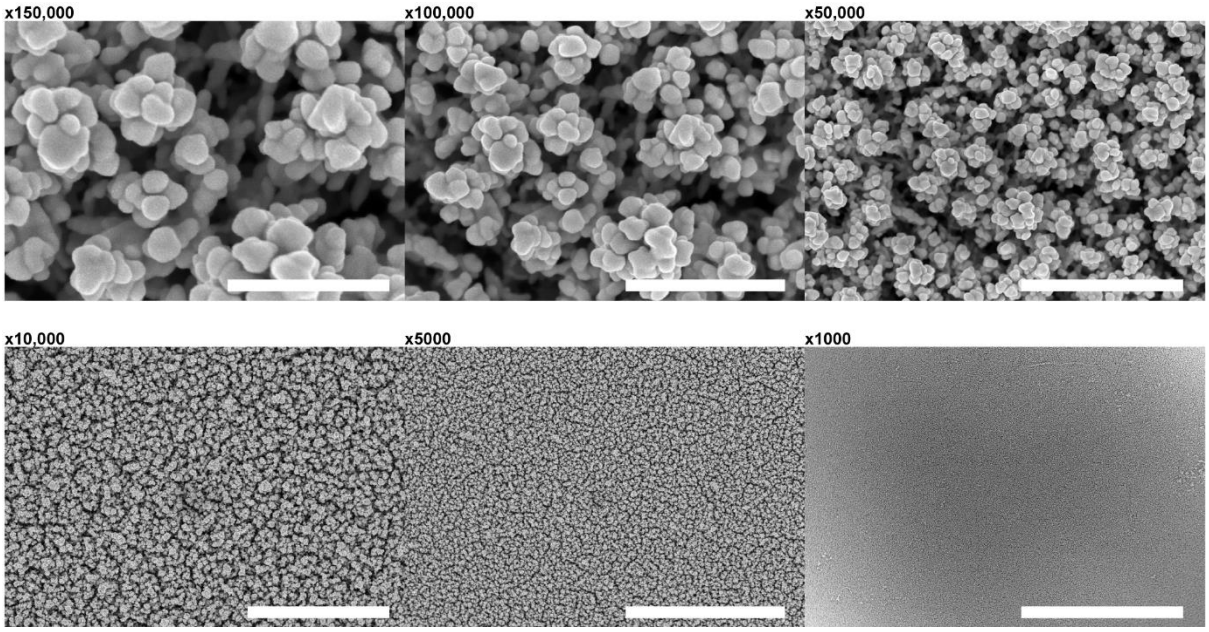


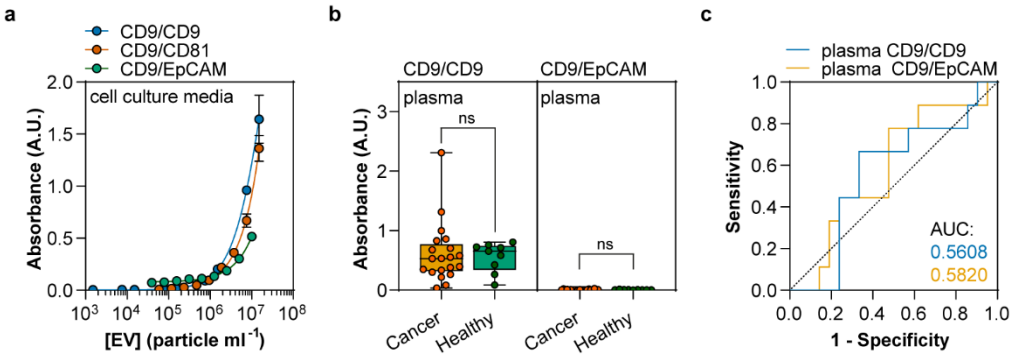
Supplementary Fig. 1 | Typical chronoamperograms, showing both cathodic and anodic currents, during the nanostructuring process conducted in photolithographic gold electrodes in different control solutions (one-component controls are all greyed out).



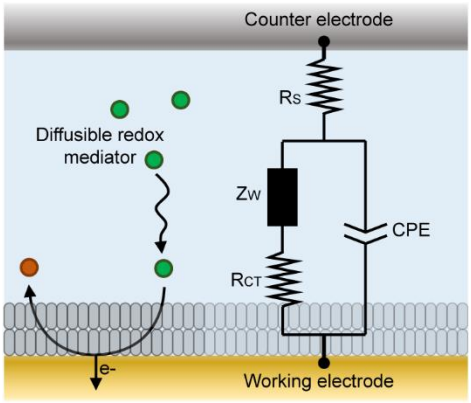
Supplementary Fig. 2 | Scanning electron micrographs of cross-sections of nanostructured gold electrodes conducted by chronoamperometry after different numbers of voltage steps. Scale bar: 2 µm.



Supplementary Fig. 3 | Scanning electron micrographs of nanostructured gold electrodes at different magnifications, displaying grain detail at higher magnifications and surface homogeneity at low magnifications (scale bars: from left to right, top to bottom: 300 nm, 500 nm, 1 µm, 5 µm, 10 µm, and 50 µm).



Supplementary Fig. 4 | Calibration plot for detection of $CD9^+$, $CD81^+$ or $EpCAM^+$ on CD9-captured extracellular vesicles from cell culture media, representing absorbance (circles) *vs*. particle concentration in traditional ELISA (n = 4 wells). Error bars represent the standard deviation of the mean. **b**, Analysis of clinical human plasma samples using ELISA with different assay schemes, detection of $CD9^+$ or $EpCAM^+$ on CD9-captured extracellular vesicles (21 cancer samples and nine healthy samples with three technical replicates for each). The boxes extend from the 25^{th} to 75^{th} percentiles, the middle line is the median, and the whiskers extend from min to max values. **c**, Characteristic curve showing the classification ability (healthy, cancer) for the two employed assays on plasma clinical samples.



Supplementary Fig. 5 | Nyquist equivalent circuit and schematic representation of each element of the circuit.

Supplementary table 1. Facet relative abundance in flat and NSG electrodes

Sample	Facet relative abundance (%)				Crystallite size (nm)
	(111)	(200)	(220)	(311)	
Flat gold	13	10	72	5	19 ± 4
NSG	17	12	63	8	21 ± 4

NSG: Nanostructured and nanoporous gold

Supplementary table 2. Other methods to generate high surface gold electrodes.

Method	Suitable for Photo-lithographic electrodes?	Acids, Toxic reagents or Solvents?	Fabrication throughput	Complexity	Time	Residues	Porosity / anti-biofouling	Ref.
EC etching	✗	✓	•	••	•••	✗	✓	1, 2
Template-free ED	✓	✗	••	••	•	✗	✗	3
Nanoporous filter template ED	✓	✓	•	•••	••	✗	✓	4, 5
Surfactant-based template ED	✓	✓	••	•••	••	✗	✗	6
EC roughening	✗	✓	••	•	•	✗	✗	7
Nanoparticles	✓	✗	••	•	•	✗	✗	8
PVD + chemical dealloying	✓	✓	•••	•••	•••	✓	✓	9
EC dealloying	✓	✓	•••	•••	•••	✗	✓	10
ED and EC dealloying	✗	✓	•	••	••	✓	✓	11
SEEDING	✓	✗	••	••	•	✗	✓	

EC: Electrochemical, **ED**: Electrodeposition, **PVD**: Physical vapor deposition (thermal evapotation or sputtering)

✗: No, ✓: Yes, •: Low, ••: Middle, •••: High. The symbols are colored in **green** when the outcome is good/positive, **red** when is bad/negative, and **grey** when is fair.

Supplementary References

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