

Supporting information

Toward environmentally favorable nano-sensing by production of reusable gold nanoparticles from gold nano-waste: Life cycle and nanocircular economy implications

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Experimental details

All chemicals used in this study were ACS reagent or molecular biology grade. Nitric acid (HNO₃), hydrochloric acid (HCl), sodium citrate tribasic dihydrate (Na₃Cit), hydrogen tetrachloroaurate hydrate (HAuCl₄), malachite green isothiocyanate (MGITC), polyethylene glycol 1,000 (1k-PEG), magnesium chloride (MgCl₂), phosphate buffered saline (PBS), tween 20 (T) were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Hampton, NH). All glassware was washed with aqua regia (3:1(v:v); HNO₃:HCl) prior to use. Nano-pure water (>18.2 M Ω · cm) is used as a solvent for solution preparation unless otherwise noted.

AuNP synthesis. For AuNP synthesis, a seed-mediated growth method were used with a reducing agents.^{1,2} Simply, seed particles (\approx 13 nm) were made by boiling 100 mL of 1 mM HAuCl₄ and 3.88 mM Na₃Cit in a round-bottom flask on a heating mantle followed by refluxing for 30 mins. For the synthesis of larger particles, 818 μ L of seed particles were put into 100 mL of 0.254 mM HAuCl₄ and 0.17 mM Na₃Cit. The particles were filtered through a 0.22 μ m PTFE filter to remove bigger particles. The concentration of nanoparticles was indirectly measured to be \sim 0.1 nM using Beer's law ($A = C \times \epsilon \times l$; A is the peak absorbance, C is the concentration, ϵ is the extinction coefficient, l is the optical path length). The absorbance spectra were measured by use of a UV-Vis spectrophotometer (Cary 5000 UV-Vis-NIR Spectrophotometer, Agilent, Santa Clara, CA).

Functionalization of AuNPs with probes. Following synthesis, the AuNP surface was functionalized with one of two aptamers that were complementary to target single-stranded DNA (ssDNA; **Probe A** and **B**). In this study, the *int11* gene was targeted as a model ssDNA. *int11* segment was determined from the identified *int11* from Blast Local Alignment Search Tool (BLAST) using forward and reverse primers. Each probe contains a thiol group for improved

affinity to the metal surface, a 20-thymine spacer, and complementary gene sequence to the *intI1* segment. All aptamers and target ssDNA were purchased from Integrated DNA Technologies, Inc. (IDT, Coralville, IA). Their sequences are listed as follows:

Target ssDNA:

GTGCACGGGCATGGTGGCTGAAGGACCAGGCCGAGGGCCGCAGCGGGCGTTGCGCTTCC
CGACGCCCTTGAGCGGAAGTATCCGCGC

Probe A: SH-T₂₀*-TCAGCCACCATGCCCGTGCAC

Probe B: SH-T₂₀*-TGCGGCCCTCGGCCTGGTCCT

*T₂₀: sequential 20-thymine as a spacer

Color matches with complementary genes

Probe A and B contain a thiol group at the 5' end which has high affinity to the metal surface. Probes were kept and activated in Tris-EDTA (TE) buffer with 50 mM Dithiothreitol (DTT) until use. Prior to use, probes were subjected to a Nap-10 column (GE Healthcare, Chicago, IL) twice for purification. An excess of probes (10,000 probes per NP) was added into 1 mL of 0.1 nM as-synthesized AuNPs suspension in a 2-mL centrifuge tube. To maintain monomeric NPs, 200 μ L of MGITC was added and incubated for 30 mins at room temperature (300 molecules per NP). Following incubation, a low-pH assisted method proven to be capable of rapid and high loading was applied using Na₃Cit (pH adjusted to 3 using HCl).³ Small aliquots of 250 mM Na₃Cit were added into the sample 4 \times to make it 30 mM and 1 hr incubation afterward. Five μ L of 1k-PEG was added into the sample for stabilization and diluted with PBS and 0.1% Tween 20 (PBS-T20), washed by centrifugation (3000 \times g for 15 mins) and resuspended in PBS-T 3 \times to remove excess chemicals and DNA other than functionalized NPs. Then, two types of functionalized AuNPs were prepared.

Detection assay. Two different types of 10 pM functionalized AuNPs and ssDNA were added into 1 mL of hybridization buffer (5% formamide, 4% dextran sulfate, and 5 mM MgCl₂). After denaturing at 95 °C for 5 mins, the probes were allowed to hybridize with *intI1* gene segment and the absorbance spectrum was then monitored using a UV-Vis spectrophotometer. Following

hybridization, the distance between AuNPs decreased in the presence of ssDNA, leading to the shift of absorbance peak to a longer wavelength.

Detection of ssDNA using aptamer-functionalized AuNPs

In this study, AuNPs were synthesized using the seed-mediated growth method. Once the small-sized Au nano-seeds were made, additional Au salt was added to their surface in the presence of the reducing agent, Na₃Cit. As-synthesized AuNPs were functionalized with thiolated aptamers complementary to *int11*. **Figure S1A** illustrates the normalized absorbance spectra of Au nano-seeds, AuNPs, and aptamer-functionalized AuNPs (Probe A and B) measured using a UV-Vis spectrophotometer. From Au nano-seeds to the final AuNPs, the absorbance peak shifted from 513 to 538 nm, implying the diameter of particles became bigger. **Figure S1B** summarizes the AuNP size distribution (n=200) determined via ImageJ (National Institutes of Health, NIH) analysis of collected transmission electron microscopy (TEM) images (a representative image is provided in the inset) along with a photograph showing the pink color of the AuNP suspension. The AuNPs were quasi-spherical and exhibited a diameter of 42.9 (\pm 6.2) nm. There was minimal red-shift in the absorbance peak wavelength following aptamer functionalization thus demonstrating successful aptamer attachment to the surface and minimal aggregation of the functionalized AuNPs. Additionally, the dispersive stability of colloidal AuNPs in 50 μ M Na₃Cit solution was confirmed by the negative charge of electrophoretic mobility (EM) and zeta-potential (-3.1×10^{-8} m²/Vs and -34.8 mV), measured using dynamic light scattering (DLS).

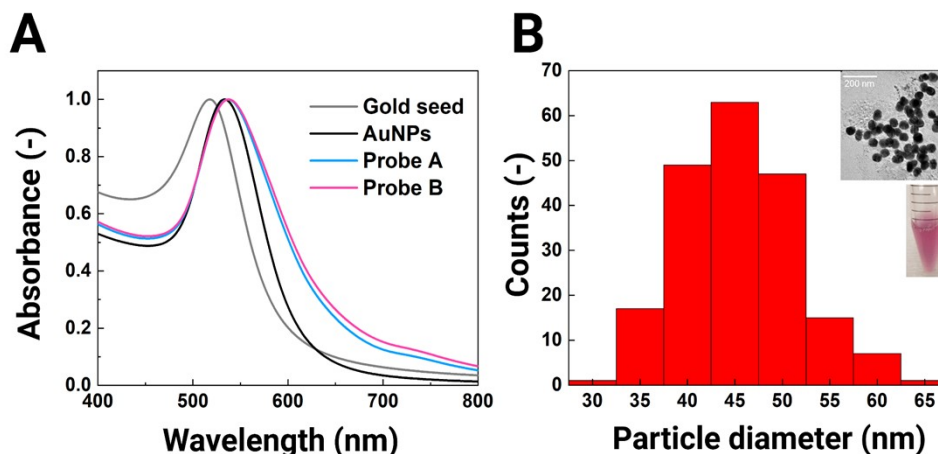


Figure S1 (A) Normalized absorbance spectra of Au seed, AuNPs, and aptamer-functionalized AuNPs by the absorbance at the peak. (B) The size distribution of as-synthesized AuNPs. Insets showed the TEM picture of AuNPs and the apparent color of AuNP suspension.

For colorimetric detection, the absorbance spectra of two nanoprobe in hybridization buffer were monitored for 48 hrs in the presence of *intI1* with $10\times$ serial dilution from 10 nM to 10 pM (**Figure S2A**). It was observed that the higher the concentration of *intI1*, the greater the absorbance profile flattened, thus illustrating that the probes aggregated due to hybridization between *intI1* and the probes. Lower concentrations of *intI1* limited the overall extent of aggregation. For 100 and 10 pM *intI1*, there was little decrease in the peak absorbance and a slight increase at the tail because of the formation of the bigger-sized Au aggregates by hybridization.^{4,5} **Figure S2B** shows how the absorbance changed over time. It increased for 48 hrs. However, the clear differences of the absorbance change with different *intI1* concentrations were observed within ~4-8 hrs, demonstrating rapid confirmation of hybridize-induced aggregation.

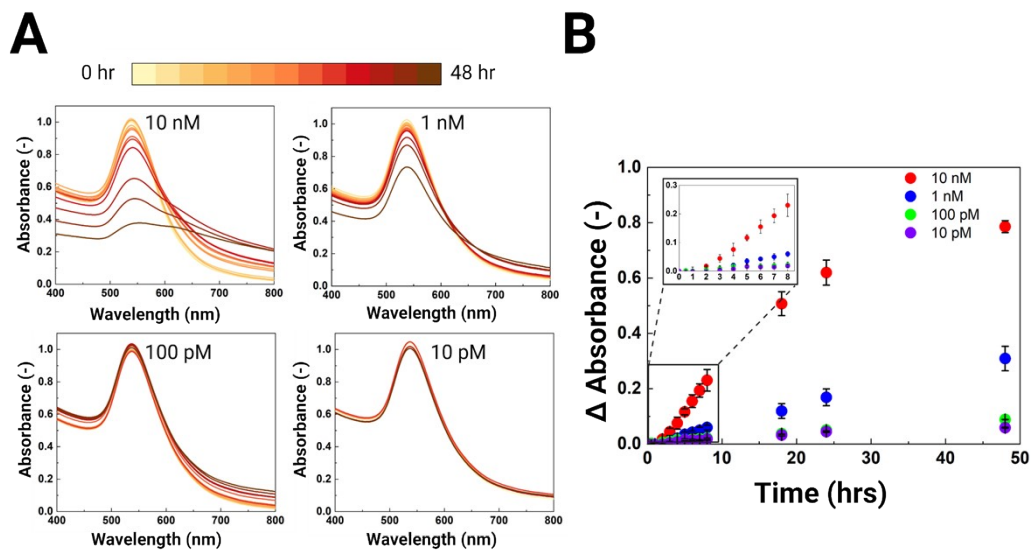


Figure S2 (A) Monitoring the absorbance spectra of Au nanoprobe aggregates with hybridization-induced aggregation in the presence of *int11* gene segments with different concentrations from 10 nM to 10 pM. (B) The absorbance change kinetics. The inset plot showed a short period. The symbol and error bar showed the mean and standard deviation of triplicates.

Simulation of Au nano-waste reuse details

The reuse of Au nano-waste can be achieved through recovery and recycling. Additional chemicals and energy demands are required for the recovery and recycling of Au nano-waste and thus their environmental impacts need to be evaluated before widespread adoption. Here, we simulated two recovery methods for Au nano-waste. The first method uses α -CD to selectively recover Au^{3+} , requiring various chemicals for the process: precipitation (sodium chloride (NaCl) and sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$)), dissolution (hydrobromic acid (HBr), nitric acid (HNO_3), nano-pure water), and selective complexation (α -CD). Second, the thermo-reversible CPE method uses a nonionic surfactant (Triton X-114) for AuNP recovery. Triton X-114 at a concentration above the critical micelle concentration (CMC) was added and heated to 40°C (temperature above the lower critical solution temperature (LCST)) to capture AuNPs. This process requires NaCl for precipitation. After the recovery process, we simulated the recycling of the recovered Au by

dissolution in a strong acid solution (aqua regia; a mixture of HNO_3 and HCl at a volume ratio of 3:1). The residual HNO_3 is boiled off with intermittent addition of HCl addition. Lastly, pH is adjusted using potassium hydroxide (KOH).

Table S1 Materials with the required amount in units for synthesis process. Corresponding inputs chosen from the EcoInvent database (v3.5) in SimaPro (v9.0). Functional unit = 100 femtomoles of AuNPs (= 100 pM × 1 mL)

Process	Materials	Amount	Unit	Corresponding LCI unit process	Ref.
Synthesis	Chloroauric acid	0.089	mg	Custom defined, see Table S6	
	Trisodium citrate	0.044	mg	Custom defined, see Table S6	
	Electricity (Heating)	4.439	kJ	Electricity, medium voltage {US} market group for APOS, S	6
	Electricity (Stirring)	0.555	kJ	Electricity, medium voltage {US} market group for APOS, S	6
	Tap water	1415.363	mg	Tap water {GLO} market group for APOS, S	6
	Nano-pure water	24.693	g	Water, ultrapure {GLO} market for APOS, S	6
	Hydrochloric acid (30%)	0.101	mg	Hydrochloric acid, without water, in 30% solution state {RER} market for APOS, S	6
	Nitric acid (50%)	0.040	mg	Nitric acid, without water, in 50% solution state {GLO} market for APOS, S	6

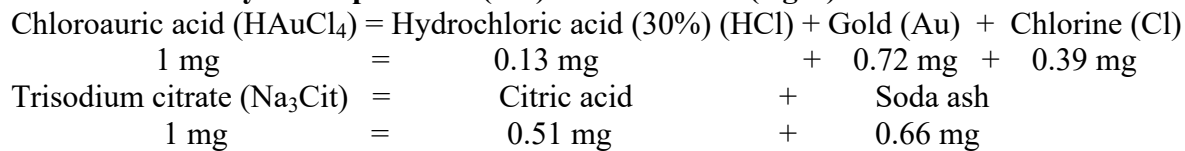
*Created inputs were customized as shown in **Table S6**.

*GLO, Global; APOS, Allocation at the point of substitution; RER, Europe; US, United States;

*Stoichiometry equations for customization of chloroauric acid and trisodium citrate are shown below

*Brief process for synthesis: after all glassware was washed with an acid solution (**hydrochloric acid (30%)** and **nitric acid (50%)**) and water (**tap** and **nano-pure water**), **chloroauric acid** and **trisodium citrate** solution in **nano-pure water** was stirred and boiled (**electricity**) in a round-bottom flask on a heating mantle.

The stoichiometry of the products (left) and reactants (right) for customization



The total amount of HAuCl₄ required for production of 100 pM colloidal AuNPs in 100 mL is [(0.818 μL × 1 mM) + (100 mL × 0.254 mM)] × 339.785 g/mol = 8.9079 mg - (A)

The total amount of Na₃Cit required for production of 100 pM colloidal AuNPs in 100 mL is [(0.818 μL × 3.88 mM) + (100 mL × 0.17 mM)] × 258.06 g/mol = 4.4058 mg - (B)

The total amount of required HAuCl₄ for functional unit = 0.01 × (A) = 0.089 mg

The total amount of required Na₃Cit for functional unit = 0.01 × (B) = 0.044 mg

Table S2 Materials with the required amount in units for functionalization process. Corresponding inputs chosen from the EcoInvent database (v3.5) in SimaPro (v9.0). Functional unit = 100 femtomoles of AuNPs (= 100 pM × 1 mL)

Process	Materials	Amount	Unit	Corresponding LCI unit process	Ref.
Functionalization	Thiol Modifier C6-S-S	0.00033	mg	Custom defined, see Table S6	
	ssDNA	0.0310	mg	Custom defined, see Table S6	
	TE Buffer	100	mg	Custom defined, see Table S6	
	Trisodium citrate	0.5161	mg	Custom defined, see Table S6	
	Hydrochloric acid (30%)	0.0280	mg	Hydrochloric acid, without water, in 30% solution state {RER} market for APOS, S	
	MGITC	0.00001185	mg	Custom defined, see Table S6	
	1k-PEG	0.005	mg	Ethylene glycol {GLO} market for APOS, S	
	Electricity	0.0108	kJ	Electricity, medium voltage {US} market group for APOS, S	7,8
	Sodium chloride	24.018	mg	Sodium chloride, powder {GLO} market for APOS, S	
	Potassium chloride	0.604	mg	Potassium chloride, as K ₂ O {GLO} market for APOS, S	
	Disodium phosphate	3.408	mg	(Substituted to) Trisodium phosphate {GLO} market for trisodium phosphate APOS, S	
	Potassium dihydrogen phosphate	0.817	mg	(Substituted to) Trisodium phosphate {GLO} market for trisodium phosphate APOS, S	
	Tween 20	0.3	mg	(Substituted to) Non-ionic surfactant {GLO} market for non-ionic surfactant APOS, S	
	Nano-pure water	3000	mg	Water, ultrapure {GLO} market for APOS, S	

*Created inputs were customized as shown in **Table S6**.

*GLO, Global; APOS, Allocation at the point of substitution; RER, Europe; US, United States; TE, Tris-EDTA; MGITC, Malachite green isothiocyanate

*Brief process: probes containing a thiol group at the 5' end (**thiol modifier C6-S-S**) were kept and activated in **Tris-EDTA (TE) buffer**. Probes (10,000 probes per NP) were added into AuNPs suspension. To maintain monomeric NPs, **MGITC** was added and incubated. Following incubation, a low-pH trisodium citrate (**trisodium citrate** and **hydrochloric acid (30%)**) were added. **1k-PEG** was added into the sample for stabilization and diluted with PBS (**sodium chloride**, **potassium chloride**, **disodium phosphate**, **potassium dihydrogen phosphate**, and **nano-pure water**) and **Tween 20** (PBS-T20), washed by centrifugation (**electricity**) and resuspended in PBS-T20.

Table S3 Materials with the required amount in units for detection assay process. Corresponding inputs chosen from the EcoInvent database (v3.5) in SimaPro (v9.0). Functional unit = 100 femtomoles of AuNPs (= 100 pM × 1 mL)

Process	Materials	Amount	Unit	Corresponding LCI unit process	Ref.
Detection assay	Formic acid	0.0511	mg	Formic acid {RER} market for APOS, S	
	Ammonia	0.0189	mg	Ammonia, liquid {RER} market for APOS, S	
	Dextran	0.0198	mg	(Substituted to) Glucose {GLO} market for glucose APOS, S	
	Disodium sulfate	0.0313	mg	(Substituted to) Sodium sulfide {GLO} market for APOS, S	
	Magnesium	0.0243	mg	Magnesium {GLO} market for APOS, S	
	Chlorine	0.0707	mg	Chlorine, gaseous {RER} market for APOS, S	
	Nano-pure water	1000.0	mg	Water, ultrapure {GLO} market for APOS, S	
	Electricity	0.39748	kJ	Electricity, medium voltage {US} market group for APOS, S	

*GLO, Global; APOS, Allocation at the point of substitution; RER, Europe; US, United States;

*Brief process: Two functionalized AuNPs were added into hybridization buffer (**formic acid, ammonia, dextran, disodium sulfate, magnesium, chloride, and nano-pure water**). After denaturing at 95 °C for 5 mins (**electricity**), the probes were allowed to hybridize with target gene segment and the absorbance spectrum was then monitored using a UV-Vis spectrophotometer.

Table S4 Materials with the required amount in units for recovery processes. Corresponding inputs chosen from the EcoInvent database (v3.5) in SimaPro (v9.0). Functional unit = 100 femtomoles of AuNPs (= 100 pM × 1 mL)

Process	Materials	Amount	Unit	Corresponding LCI unit process	Ref.
Alpha-CD recovery	Sodium chloride	0.4118	mg	Sodium chloride, powder {GLO} market for APOS, S	
	Hydrobromic acid	37.7803	mg	Custom defined, see Table S6	
	Potassium hydroxide	8.2380	mg	Potassium hydroxide {GLO} market for APOS, S	
	Nitric acid (50%)	12.0021	mg	Nitric acid, without water, in 50% solution state {GLO} market for APOS, S	
	Nano-pure water	338.081229	mg	Water, ultrapure {GLO} market for APOS, S	
	Alpha-CD	9.728E-08	mg	Custom defined, see Table S6	
	Sodium thiosulfate	7.7108	mg	(Substituted to) Sodium hydrogen sulfite {GLO} market for APOS, S	
	Electricity	0.2336	kJ	Electricity, medium voltage {US} market group for APOS, S	
Triton X-114 recovery	Sodium chloride	1.9870	mg	Sodium chloride, powder {GLO} market for APOS, S	
	Triton X-114	33.0579	mg	Custom defined, see Table S6	
	Nano-pure water	1183.28375	mg	Water, ultrapure {GLO} market for APOS, S	
	Electricity	0.0072	kJ	Electricity, medium voltage {US} market group for APOS, S	7,8
	Electricity	1.6736	kJ	Electricity, medium voltage {US} market group for APOS, S	

*Created inputs were customized as shown in **Table S6**.

*GLO, Global; APOS, Allocation at the point of substitution; RER, Europe; US, United States;

*Brief process for Alpha-CD recovery: precipitation (**sodium chloride** and **sodium thiosulfate**), dissolution (**hydrobromic acid** and **nitric acid (50%)**), and **nano-pure water**) with stirring (**electricity**), and selective complexation (α -CD).

*Brief process for Triton X-114 recovery: **Triton X-114** and **sodium chloride** for precipitation were added and heated to 40 °C with stirring (**electricity**).

Table S5 Materials with the required amount in units for recycling process. Corresponding inputs chosen from the EcoInvent database (v3.5) in SimaPro (v9.0). Functional unit = 100 femtomoles of AuNPs (= 100 pM × 1 mL)

Process	Materials	Amount	Unit	Corresponding LCI unit process	Ref.
Recycling	Hydrochloric acid (30%)	25.1379	mg	Hydrochloric acid, without water, in 30% solution state {RER} market for APOS, S	
	Nitric acid (50%)	10.0082	mg	Nitric acid, without water, in 50% solution state {GLO} market for APOS, S	
	Hydrochloric acid (30%)	33.5262	mg	Hydrochloric acid, without water, in 30% solution state {RER} market for APOS, S	
	Electricity	3.8844	kJ	Electricity, medium voltage {US} market group for APOS, S	
	Potassium hydroxide	0.4118	mg	Potassium hydroxide {GLO} market for APOS, S	
	Nano-pure water	536.46853	mg	Water, ultrapure {GLO} market for APOS, S	

*Created inputs were customized as shown in **Table S6**.

*GLO, Global; APOS, Allocation at the point of substitution; RER, Europe; US, United States;

*Brief process: dissolution in aqua regia (**hydrochloric acid (30%)**, **nitric acid (50%)**, **nano-pure water**), boiling off with intermittent addition of acid solution (**electricity** and **hydrochloric acid (30%)**), and pH adjustment (**potassium hydroxide**).

Table S6 1 mg of customized chemicals from available EcoInvent database (v3.5) in SimaPro (v9.0).

Process	Materials	Amount	Unit	Corresponding LCI unit process	Ref.
Chloroauric acid	Hydrochloric acid (30%)	0.13	mg	Hydrochloric acid, without water, in 30% solution state {RER} market for APOS, S	
	Gold	0.72	mg	Gold {US} production APOS, S	
	Chlorine	0.39	mg	Chlorine, gaseous {RER} market for APOS, S	
Trisodium citrate	Citric acid	0.51	mg	Citric acid {GLO} market for APOS, S	
	Soda ash	0.66	mg	Soda ash, light, crystalline, heptahydrate {GLO} market for APOS, S	
Thiol ssDNA	Thiol Modifier C6 S-S	0.506	mg	Custom defined, see below	
	Adenine	0.046	mg	Custom defined, see below	
	Cytosine	0.038	mg	Custom defined, see below	
	Guanine	0.052	mg	Custom defined, see below	
	Thymine	0.043	mg	Custom defined, see below	
	Sodium phosphate	0.225	mg	Sodium phosphate {GLO} market for APOS, S	
	Deoxyribose	0.247	mg	(Substituted to) Glucose {GLO} market for glucose APOS, S	
Thiol Modifier C6 S-S	Hexane	0.491	mg	Hexane {GLO} market for APOS, S	
	Sulfur	0.183	mg	Sulfur {GLO} market for APOS, S	
	Sodium phosphate	0.467	mg	Sodium phosphate {GLO} market for APOS, S	
Adenine	Imidazole	0.504	mg	Imidazole {GLO} market for APOS, S	
	Pyridazine	0.593	mg	Pyridazine-compound {GLO} market for APOS, S	
	Ammonia	0.126	mg	Ammonia, liquid {RER} market for APOS, S	
Guanine	Imidazole	0.45	mg	Imidazole {GLO} market for APOS, S	
	Pyridazine	0.53	mg	Pyridazine-compound {GLO} market for APOS, S	
	Ammonia	0.113	mg	Ammonia, liquid {RER} market for APOS, S	
	Oxygen	0.106	mg	Oxygen, liquid {RER} market for APOS, S	
Cytosine	Pyridazine	0.721	mg	Pyridazine-compound {GLO} market for APOS, S	
	Ammonia	0.153	mg	Ammonia, liquid {RER} market for APOS, S	
	Oxygen	0.144	mg	Oxygen, liquid {RER} market for APOS, S	

Thymine	Pyridazine	0.635	mg	Pyridazine-compound {GLO} market for APOS, S	
	Ammonia	0.127	mg	Ammonia, liquid {RER} market for APOS, S	
	Oxygen	0.241	mg	Oxygen, liquid {RER} market for APOS, S	
TE Buffer (0.1 mL)	Methane	0.0160	mg	Methane, 96% by volume {RoW} market for methane, 96% by volume APOS, S	
	Methanol	0.0960	mg	Methanol {GLO} market for APOS, S	
	Ammonia	0.0171	mg	Ammonia, liquid {RER} market for APOS, S	
	Hydrochloric acid (30%)	0.1220	mg	Hydrochloric acid, without water, in 30% solution state {RER} market for APOS, S	
	EDTA	0.0315	mg	EDTA, ethylenediaminetetraacetic acid {GLO} market for APOS, S	
	Sodium	0.0025	mg	Soda ash, light, crystalline, heptahydrate {GLO} market for APOS, Soda	
	Sodium dithionite	0.1739	mg	Sodium dithionite, anhydrous {GLO} market for APOS, S	
	Penta-erythritol	0.1360	mg	Pentaerythritol {GLO} market for APOS, S	
	Nano-pure water	100	mg	Water, ultrapure {GLO} market for APOS, S	
Malachite Green isothiocyanate (MGITC)	Triphenyl phosphate	0.838	mg	Triphenyl phosphate {GLO} market for triphenyl phosphate APOS, S	
	Nitrogen	0.072	mg	Nitrogen, liquid {RER} market for APOS, S	
	Methane	0.165	mg	Methane, 96% by volume {RoW} market for methane, 96% by volume APOS, S	
	Ammonium thiocyanate	0.195	mg	Ammonium thiocyanate {GLO} market for APOS, S	
	Sodium perchlorate	0.314	mg	Sodium perchlorate {GLO} market for APOS, S	
Hydrobromic acid	Phosphorus	0.13	mg	Phosphorus, white, liquid {GLO} market for APOS, S	
	Bromine	0.99	mg	Bromine {GLO} market for APOS, S	
	Tap water	0.22	mg	Tap water {GLO} market group for APOS, S	
Alpha-CD	Potato starch	1.67	mg	Potato starch {GLO} market for APOS, S	
	Nano-pure water	16.67	mg	Water, ultrapure {GLO} market for APOS, S	
	Electricity	180	kJ	Electricity, medium voltage {US} market group for	

	(Heating)			APOS, S	
	Electricity (Stirring)	20	kJ	Electricity, medium voltage {US} market group for APOS, S	
Triton X-114	Propane	0.164	mg	Propane {GLO} market for APOS, S	
	Phenol	0.175	mg	Phenol {GLO} market for APOS, S	
	Ethylene glycol	0.809	mg	Ethylene glycol {GLO} market for APOS, S	

*GLO, Global; APOS, Allocation at the point of substitution; RER, Europe; US, United States

*Stoichiometry equations for customization of thiol ssDNA, thiol modifier, ssDNA, TE buffer, MGITC, 1k-PEG, PBS-T20, hybridization buffer, hydrobromic acid, alpha-CD, and Triton X-114 are shown below

The stoichiometry of the products (left) and reactants (right) for customization

$$\text{Thiol ssDNA (711.06 g/mol)} = \text{Thiol modifier (351.09 g/mol)} + \text{ssDNA (359.97 g/mol)}$$
$$1 \text{ mg} = 0.494 \text{ mg} + 0.506 \text{ mg}$$

$$\text{Thiol modifier (351.09 g/mol)} = 2 \text{ Hexane (86.18 g/mol)} + 2 \text{ S (32.07 g/mol)} + \text{Sodium phosphate (163.94 g/mol)}$$
$$1 \text{ mg} = 0.491 \text{ mg} + 0.183 \text{ mg} + 0.467 \text{ mg}$$

$$\text{ssDNA (359.97 g/mol)} = 86 \text{ bps} * \left\{ \left(\frac{1}{4} \right) \text{ Adenine} + \left(\frac{1}{4} \right) \text{ Guanine} + \left(\frac{1}{4} \right) \text{ Cytosine} + \left(\frac{1}{4} \right) \text{ Thymine} \right. \\ \left. + \text{Sodium phosphate (163.94 g/mol)} + \text{Glucose (180.156 g/mol)} \right\}$$
$$1 \text{ mg} = 0.094 \text{ mg} + 0.105 \text{ mg} + 0.077 \text{ mg} + 0.088 \text{ mg} + 0.455 \text{ mg} + 0.500 \text{ mg}$$

$$\text{Adenine (135.13 g/mol)} = \text{Imidazole (68.077 g/mol)} + \text{Pyridazine (80.09 g/mol)} + \text{NH}_3 (17.031 \text{ g/mol})$$
$$1 \text{ mg} = 0.504 \text{ mg} + 0.593 \text{ mg} + 0.126 \text{ mg}$$

$$\text{Guanine (151.13 g/mol)} = \text{Imidazole (68.077 g/mol)} + \text{Pyridazine (80.09 g/mol)} + \text{NH}_3 (17.031 \text{ g/mol}) + \left(\frac{1}{2} \right) \text{O}_2 (32 \text{ g/mol})$$
$$1 \text{ mg} = 0.450 \text{ mg} + 0.53 \text{ mg} + 0.113 \text{ mg} + 0.106 \text{ mg}$$

$$\text{Cytosine (111.1 g/mol)} = \text{Pyridazine (80.09 g/mol)} + \text{NH}_3 (17.031 \text{ g/mol}) + \left(\frac{1}{2} \right) \text{O}_2 (32 \text{ g/mol})$$
$$1 \text{ mg} = 0.721 \text{ mg} + 0.153 \text{ mg} + 0.144 \text{ mg}$$

$$\text{Thymine (126.11 g/mol)} = \text{Pyridazine (80.09 g/mol)} + \text{CH}_4 (16.04 \text{ g/mol}) + \text{O}_2 (32 \text{ g/mol})$$
$$1 \text{ mg} = 0.635 \text{ mg} + 0.127 \text{ mg} + 0.254 \text{ mg}$$

Deoxyribose = Glucose

Phosphate = Sodium phosphate

TE buffer 0.1 mL = 10 mM Tris + Hydrochloric acid (30%) + 1 mM disodium EDTA + 10 mM DTT + 0.1 mL nano-pure water

$$0.1 \text{ mL} = 0.121 \text{ mg} + 0.122 \text{ mg} + 0.034 \text{ mg} + 0.154 \text{ mg}$$

$$\text{Tris (121.136 g/mol)} = \text{CH}_4 (16.04 \text{ g/mol}) + 3 \text{ Methanol (32.04 g/mol)} + \text{NH}_3 (17.031 \text{ g/mol})$$
$$1 \text{ mg} = 0.132 \text{ mg (0.24 cm}^3\text{)} + 0.793 \text{ mg} + 0.141 \text{ mg}$$

$$\text{Disodium EDTA (315.23 g/mol)} = \text{EDTA (292.24 g/mol)} + 2 \text{ Na (22.99 g/mol)}$$
$$1 \text{ mg} = 0.927 \text{ mg} + 0.073 \text{ mg}$$

$$\text{DTT (154.253 g/mol)} = \text{Sodium dithionite (174.107 g/mol)} + \text{Penta-erythritol (136.15 g/mol)}$$
$$1 \text{ mg} = 1.129 \text{ mg} + 0.883 \text{ mg}$$

Malachite Green isothiocyanate (MGITC) (389.382 g/mol) = Triphenyl phosphate (326.28 g/mol) + N₂ (28.01 g/mol) + 4 CH₄ (16.04 g/mol) + Ammonium thiocyanate (76.122 g/mol) + Sodium perchlorate (122.44 g/mol)

$$1 \text{ mg} = 0.838 \text{ mg} + 0.072 \text{ mg} + 0.165 \text{ mg (0.29 cm}^3\text{)} + 0.195 \text{ mg} + 0.314 \text{ mg}$$

1k -polyethylene glycol (1k-PEG) (993.12 g/mol) = 16 * Ethylene glycol (62.07 g/mol)

PBS-T20 3 mL = 137 mM NaCl + 2.7 mM KCl + 8 mM Na₂HPO₄ + 2 mM KH₂PO₄ + 0.01% Tween-20 + 3 mL nanopure water

$$3 \text{ mL} = 24.018 \text{ mg} + 0.604 \text{ mg} + 3.408 \text{ mg} + 0.817 \text{ mg} + 0.3 \text{ mg} + 3 \text{ mg}$$

$$\text{Na}_2\text{HPO}_4 (141.96 \text{ g/mol}) = \text{Trisodium phosphate (163.94 g/mol)}$$

$$\text{KH}_2\text{PO}_4 = \text{Trisodium phosphate (163.94 g/mol)}$$

Tween 20 = Non-ionic surfactant

Hybridization buffer (100 fmoles in 1 mL) = 5% formamide + 4% dextran sulfate + 1mM MgCl₂ (95.211 g/mol) + 1 mL nano pure water

$$1 \text{ mL} = 0.05 \text{ mg} + 0.04 \text{ mg} + 0.095 \text{ mg} \text{ (} 0.001 \text{ mol/L} * 0.001 \text{ L} * 95.211 \text{ g/mol} * 1000 \text{ mg/g)}$$

$$\text{MgCl}_2 \text{ (} 95.211 \text{ g/mol)} = \text{Magnesium (} 24.305 \text{ g/mol)} + 2 \text{ Chlorine (} 35.453 \text{ g/mol)}$$

$$1 \text{ mg} = 0.256 \text{ mg} + 0.744 \text{ mg}$$

$$\text{Dextran sulfate monoisotopic mass (} 725.905 \text{ g/mol)} = 2 \text{ Glucose (} 180.156 \text{ g/mol)} + 4 \text{ Na}_2\text{SO}_4 \text{ (} 142.04 \text{ g/mol)}$$

$$\text{Dextran sulfate (} 500,148.545 \text{ g/mol)} = 1378 \text{ Glucose (} 180.156 \text{ g/mol)} + 2756 \text{ Na}_2\text{SO}_4 \text{ (} 142.04 \text{ g/mol)}$$

$$1 \text{ mg} = 0.496 \text{ mg} + 0.783 \text{ mg}$$

$$\text{Formamide (} 45.04 \text{ g/mol)} = \text{formic acid (} 46.03 \text{ g/mol)} + \text{NH}_3 \text{ (} 17.031 \text{ g/mol)}$$

$$1 \text{ mg} = 1.022 \text{ mg} + 0.378 \text{ mg}$$

$$\text{Hydrobromic acid} = \text{Phosphorous} + \text{Bromine} + \text{Tap water}$$

$$1 \text{ mg} = 0.13 \text{ mg} + 0.99 \text{ mg} + 0.22 \text{ mg}$$

$$\text{Alpha-CD} = \text{Potato starch} + \text{Water}$$

$$1 \text{ mg} = 1.67 \text{ mg} + 16.67 \text{ mg}$$

$$\text{Triton X-114 (} 537 \text{ g/mol)} = 2 \text{ Propane (} 44.1 \text{ g/mol)} + \text{Phenol (} 94.11 \text{ g/mol)} + 7 * \text{Ethylene glycol (} 62.07 \text{ g/mol)}$$

$$1 \text{ mg} = 0.164 \text{ mg} + 0.175 \text{ mg} + 0.809 \text{ mg}$$

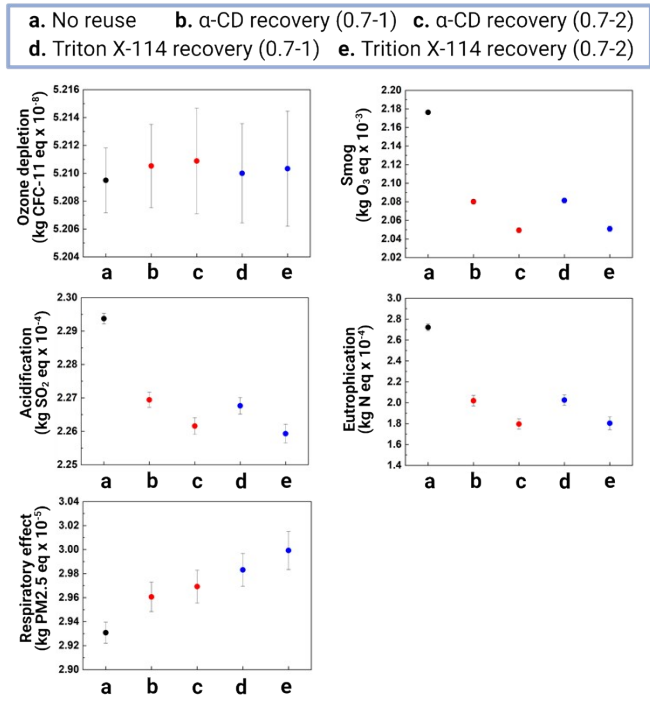


Figure S3 Uncertainty analysis of environmental impacts (Ozone depletion, smog, acidification, eutrophication, respiratory effect). No reuse scenario and two recovery method-mediated reuse scenarios (α -CD and Triton X-114) with the recovery efficiency of 0.7 and 1-2 reuse cycles were compared.

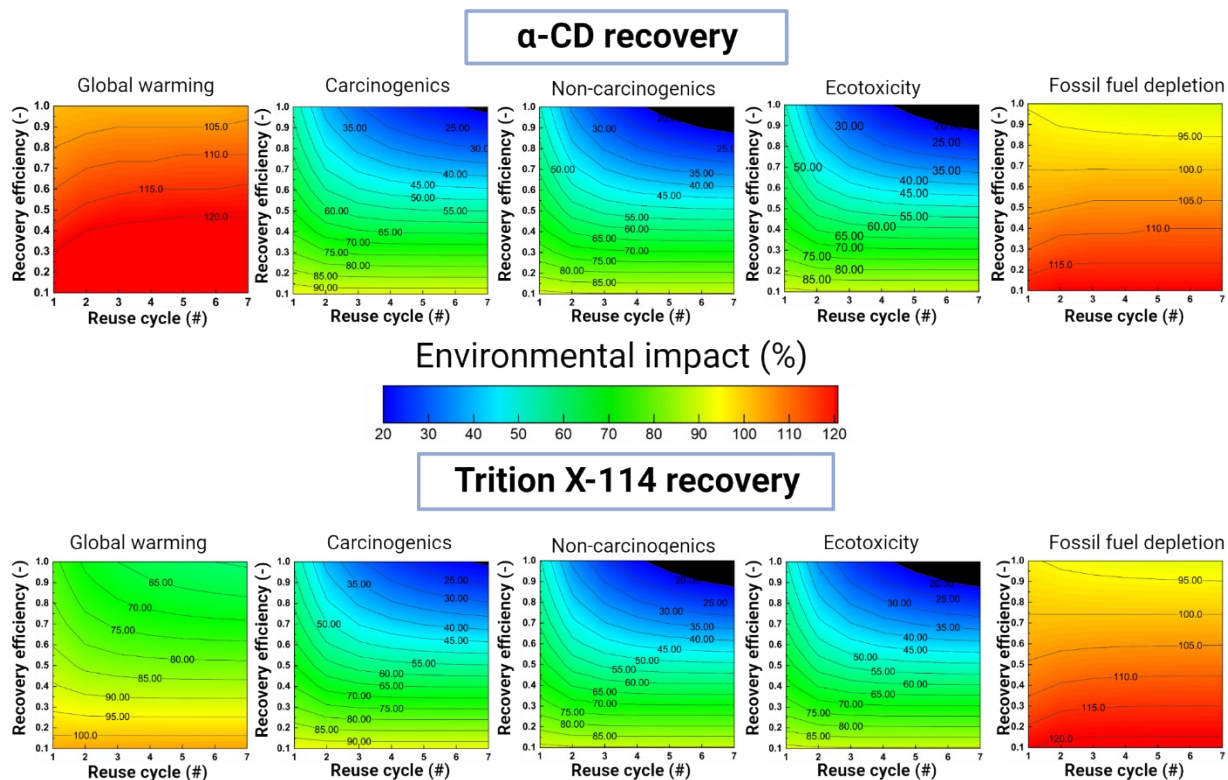


Figure S4 Sensitive analysis of environmental impacts (global warming, carcinogenic, non-carcinogenic, ecotoxicity, fossil fuel depletion) with varying recovery efficiency (0.0 to 1.0) and the number of reuse cycles (#). The environmental impacts were normalized by the no-reuse scenario.

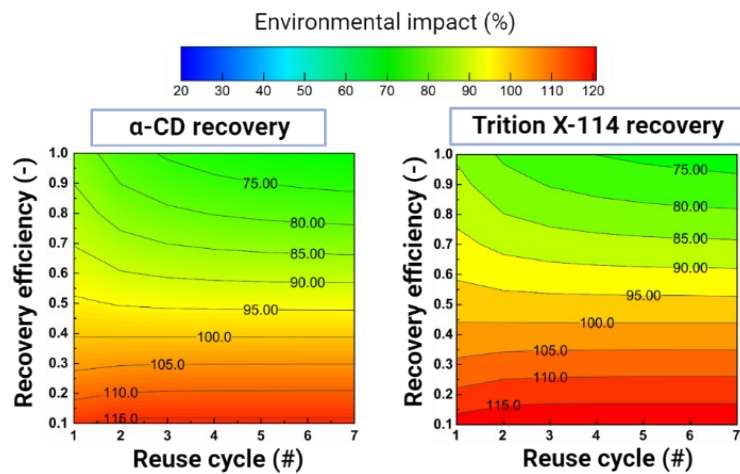


Figure S5 Sensitivity analysis of cumulative energy demand (CED) with varying recovery efficiency (0.0 to 1.0) and the number of reuse cycles (#). The CEDs were normalized by the no-use scenario.

Reference

- (1) Turkevich, J.; Stevenson, P. C.; Hillier, J. A Study of the Nucleation and Growth Processes in the Synthesis of Colloidal Gold. *Discuss. Faraday Soc.* **1951**, *11* (c), 55–75. <https://doi.org/10.1039/DF9511100055>.
- (2) FRENS, G. Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions. *Nat. Phys. Sci.* **1973**, *241* (105), 20–22. <https://doi.org/10.1038/physci241020a0>.
- (3) Zhang, X.; Servos, M. R.; Liu, J. Instantaneous and Quantitative Functionalization of Gold Nanoparticles with Thiolated DNA Using a PH-Assisted and Surfactant-Free Route. *J. Am. Chem. Soc.* **2012**, *134* (17), 7266–7269. <https://doi.org/10.1021/ja3014055>.
- (4) Bellino, M. G.; Calvo, E. J.; Gordillo, G. Adsorption Kinetics of Charged Thiols on Gold Nanoparticles. *Phys. Chem. Chem. Phys.* **2004**, *6* (2), 424–428. <https://doi.org/10.1039/b312252b>.
- (5) Westcott, S. L.; Oldenburg, S. J.; Lee, T. R.; Halas, N. J. Construction of Simple Gold Nanoparticle Aggregates with Controlled Plasmon–Plasmon Interactions. *Chem. Phys. Lett.* **1999**, *300* (5–6), 651–655. [https://doi.org/10.1016/S0009-2614\(98\)01410-9](https://doi.org/10.1016/S0009-2614(98)01410-9).
- (6) Pati, P.; McGinnis, S.; Vikesland, P. J. Waste Not Want Not: Life Cycle Implications of Gold Recovery and Recycling from Nanowaste. *Environ. Sci. Nano* **2016**, *3* (5), 1133–1143. <https://doi.org/10.1039/c6en00181e>.
- (7) Soomro, R. R.; Ndikubwimana, T.; Zeng, X.; Lu, Y.; Lin, L.; Danquah, M. K. Development of a Two-Stage Microalgae Dewatering Process – A Life Cycle Assessment Approach. *Front. Plant Sci.* **2016**, *7* (FEB2016). <https://doi.org/10.3389/fpls.2016.00113>.
- (8) Molina Grima, E.; Belarbi, E. H.; Ación Fernández, F. G.; Robles Medina, A.; Chisti, Y. Recovery of Microalgal Biomass and Metabolites: Process Options and Economics. *Biotechnol. Adv.* **2003**, *20* (7–8), 491–515. [https://doi.org/10.1016/S0734-9750\(02\)00050-2](https://doi.org/10.1016/S0734-9750(02)00050-2).