# Supporting Information

## Thermo-responsive 3D nanostructures for enhanced performance in food-poisoning bacterial analysis

*Yeonwoo Jeong[a], Jueun Kim[b], Jina Lee,[a,c], Seungbeom Seo[a,d], Seokbeom Roh[e], Gyudo Lee[e], Bong Gill Choi[b], Nam Ho Bae[f], Juyeon Jung[a,c,g], Taejoon Kang[a,g], Kyoung G. Lee[f]*, and Eun-Kyung Lim[a,c,g]**

[a]Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB) 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea

[b]Department of Chemical Engineering, Kangwon National University, Samcheok 25913, Republic of Korea

[c]Department of Nanobiotechnology, KRIBB School, University of Science and Technology (UST), 125 Gwahak-ro, Yuseong-gu, Daejeon 34113, Republic of Korea

[d]Department of Cogno-Mechatronics Engineering, Pusan National University, Pusan 46241, Republic of Korea

[e]Department of Biotechnology and Bioinformatics, Korea University, Sejong 30019, Republic of Korea

[f]Center for Nano Bio Development, National NanoFab Center (NNFC), 291 Daehak-ro, Yuseong-gu 34141, Republic of Korea

[g]School of Pharmacy, Sungkyunkwan University, Suwon 16419, Korea

## Materials

Glycinamide hydrochloride (98%), potassium carbonate ($K_2CO_3$, 99%), methacryloyl chloride (97%), benzyl acrylate (99.8%), ethyl acrylate (99.5%), butyl acrylate (99%), ascorbic acid, 2,2′-bipyridine (bpy, 99%), phosphate-buffered saline (PBS), 6-amino-1-hexanethiol hydrochloride and APTES (99%), perchloric acid (70%), ammonium persulfate (98%), and aniline (99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Copper (II) bromide ($CuBr_2$, 99%), BiBB (97%), and trimethylamine ($Et_3N$, 99%) were purchased from Alfa Aesar (Haverhill, MA, USA). Ethyl alcohol (EtOH, 99.5%), methyl alcohol (MeOH, 99.5%), dichloromethane (DCM, 99%), acetone (99%), and aluminum oxide ($AlO_2$, 99%) were purchased from Samchun Chemical Co. (Pyeongtaek, Gyeonggi-do, Korea). Norland Optical Adhesive 63 (NOA63) was purchased from Norland Optical Adhesives (Jamesburg, NJ, USA), and polyurethane (PU) was purchased from MINS-311RM, Minuta Tech. (Cheonan, Chungcheong-do, South Korea). All the reagents were of analytical grade and were not subjected to further purification. Luria–Bertani (LB) high-salt broth was purchased from MBcell (Seoul, Korea). Bacterial stocks of *Staphylococcus aureus* (ATCC 29213), *Salmonella enteritidis* (ATCC 13076), *Listeria monocytogenes* (ATCC 19111) and *Bacillus cereus* (ATCC 21768) were obtained from American Type Culture Collection (ATCC).

## Preparation of urchin-like 3D nanopillar substrate (URCHANO)

The overall process of fabricating urchin-like 3D nanostructured substrate (URCHANO) was fabricated with reference to previous reports (*Nano Converg.* 2021, **8**, 30)[1] and presented schematically in **Figure S1**. $SiO_2$ holes with a diameter of 500 nm were used as the master mold, and a mixture of NOA 63 and PU in a ratio of 70:30 wt% was used for pillar replication, which was fabricated using a photolithography process. The replicated nanopillar substrate was further coated with thin layers of Ti (20 nm) and Au (200 nm), followed by the polymerization of aniline on the coating at 4 °C for 8 h, which resulted in the growth of urchin-shaped PANI fibers on the nanopillar substrate and finally yielded URCHANO. Scanning electron microscopy (SEM) measurements were performed to investigate the surface morphologies of the Si mold, pristine pillars, and PANI on the nanopillar substrate (**Figure S2**). **Figure S2a** shows the typical morphology of a Si mold with a nanohole array used to construct a nanopillar substrate. For the construction of the nanopillar substrate, spin coating of the NOA 63 and PU mixture onto a Si mold enabled the formation of a highly ordered nanopillar array on the PET film, with a diameter of approximately 500 nm and an aspect ratio of 1:2.5 (**Figure S2b**). The SEM images in the middle and right panels of **Figure S2f and S2g** show the nanopillar substrate after Ti/Au coating and the final PANI fiber polymerization on the nanopillar substrate, respectively. As shown in **Figure S2g**, URCHANO, with it urchin-shaped nanofibers, allowed easy capture of the bacterial pathogen.

**Synthesis of MNAGA as the monomer for the thermo-responsive co-polymer**

The synthesis of MNAGA was synthesized with reference to previous reports (*Polymer*, 2017, **126**, 1–8 , *J. Mater. Chem. B*, 2017, **5**, 4926–4933.).[2,3] Glycinamide hydrochloride (5 g, 45.2

mmol) and potassium carbonate (12.5 g, 90.4 mmol) were first dissolved in deionized water (D.W.; 100 mL) and kept under 0 °C with vigorous stirring for 30 min. Subsequently, methacryloyl chloride (4.4 mL, 46.4 mmol) in cold ether (20 mL) was added dropwise into the reaction mixture (**Figure S3**). After 1 h, the reaction mixture was sealed and transferred to room temperature with continuous vigorous stirring for an additional 3 h. The reaction mixture was subjected to vacuum evaporation to remove the organic solvents and then lyophilized to remove the aqueous liquids. The lyophilized product was re-suspended in acetone (100 mL) and stirred at 40 °C for 2 h. The insoluble fraction was removed by filtration, and the acetone was removed by vacuum evaporation. Then, the obtained white powder was dissolved in a 1:4 mixture of MeOH and DCM (50 mL), and cooled down to 0 °C, kept for 30 min and white precipitate was removed. The organic solvent was vacuum-evaporated, and the residue was dissolved in acetone (40 mL) at 30 °C and recrystallized at -20 °C. The resulting white powder was filtered using cold acetone and dissolved in distilled water, and the aqueous phase was filtered and lyophilized. $^{1}$H NMR (MHz 400, $D_2O$) δ: 2.0 (3H, s), 4.0 (2H, s), 5.59 (1H, s), 5.85 (1H, s), yield: 26.5%.

**Fabrication of the thermo-responsive co-polymer coating on URCHANO**

To introduce thermo-responsiveness to URCHANO, thermo-responsive co-polymers were immobilized on URCHANO via SI-ARGET-ATRP method. First, URCHANO was cleaned with EtOH, dried under a stream of $N_2$ gas before use, and then activated by $O_2$ plasma treatment for 20 min, and immersed in an APTES solution (10 mM, EtOH, 3 mL). The amine-functionalized solid substrates, BiBB and $Et_3N$ containing glass vials (100 μL for each) were placed into the vacuum chamber and kept overnight for chemical vapor-phase deposition. The

unreacted BiBB and $Et_3N$ were removed using methanol, and the substrates were dried under a stream of $N_2$ gas. MNAGA and each of hydrophobic acrylate monomers (benzyl-, ethyl- and butyl) were co-polymerized onto the BiBB-immobilized solid substrates via SI-ARTEG-ATRP. Before use, each acrylate monomer was syringe-filtered using $AlO_2$ to remove the inhibitors. MNAGA and each acrylate monomer was dissolved in a 1:9 mixture of MeOH and deionized water (3 mL) to achieve a total concentration 0.5 M with different molar ratios (MNAGA:acrylate monomer = 100:0, 95:5, 90:10, and 80:20, **Table S1**). Then, a methanolic catalytic solution (0.4 mL) consisting of 0.5 mM $CuBr_2$ and 3 mM bpy was added into the solution of MNAGA and the acrylate monomer mixture (3 mL) and vortexed for 30 s. Next, the BiBB-immobilized solid substrates were immersed in a glass vial containing the MNAGA and acrylate monomer mixture. Then, 0.1 M ascorbic acid (0.1 mL) was added, and the vial was screw-capped. The reaction vial was heated to 45 °C to encouragement of the polymerization and prevent polymer aggregation. After 3 h, the MNAGA-based thermo-responsive polymer-coated URCHANO (thermo-URCHANO) was sequentially rinsed with methanol, ethanol, and D.W. and dried under a stream of $N_2$ gas. To confirm the presence of the polymer coating on the substrate, a hydrophobic Ti/Au-deposited PU nanopillar substrate before PANI formation (Au substrate) was coated with this polymer, and the changes in the water contact angle were analyzed. Before coating, the Au substrate was immersed in a 10 mM solution of 6-amino-1-hexanethiol hydrochloride in 3 mL EtOH for 3 h, rinsed with EtOH, and dried under a stream of $N_2$ gas. The subsequent process was identical to that described previously.

## Characterization

$^{1}$H-NMR spectra were obtained using AVANCE NEO (Bruker) 400 MHz, and $D_2O$ was used as the solvent. XPS was performed using K-alpha+ (Thermo Scientific). FT-IR spectra were obtained using an ALPHA FT-IR spectrometer with attenuated total reflectance (ATR) measurements. The static water contact angle and captive bubbles were measured using a Phoenix-MT (SEO). SEM images were obtained using a Hitachi S-4800 SEM instrument after Pt sputtering for 90 s. The focused ion beam (FIB)-SEM analysis was performed using a Helios NanoLab 600 Dual Beam instrument (FEI). AFM images were obtained using the tapping mode of Multimode 8 (Bruker) with RTESPA-300 probe (Bruker) and analysis by using Nanoscope Analysis (version 2.0m Bruker) and MountainsSPIP (version 9, Digital Surf.)

## Evaluation of bacterial capture and release by the thermo-responsiveness of Thermo-URCHANO

*S. aureus* was cultured in LB broth at 37 °C for 12–18 h in a shaking incubator. The CFU value (CFU/mL) of the bacterial suspension was measured by measuring the optical density at 600 nm (OD 600) using an ultraviolet (UV) spectrometer and diluting with fresh LB broth. To prepare bacterial suspensions of different concentrations, each suspension was concentrated by centrifugation at 13,500 rpm for 1 min and then re-suspended in LB broth (50 μL). Subsequently, the bacterial suspension was dropped onto a 0.5 cm (width) × 0.5 cm (length) slab of Thermo-URCHANO to capture bacteria. After 30 min, the substrate was washed with 1 mL of PBS to remove uncaptured bacteria. Each substrate was then incubated at 37 °C or 25 °C for 10 min to release the captured bacteria, and then vortexed for 10 s. The released bacteria

were concentrated by centrifugation at 13,500 rpm for 1 min, after which genomic DNA (gDNA) was extracted using the AccuPrep® Genomic DNA Extraction Kit (Bioneer). The bacterial capture and release performance of Thermo-URCHANO in response to temperature changes was evaluated by measuring gDNA concentrations using real-time PCR (qPCR). The qPCR reaction mixture was prepared using forward and reverse primers (2 μL each), nuclease-free water (4 μL), QuantiTect SYBR Green PCR kit (10 μL; Qiagen), and the extracted gDNA (2 μL). The primers were designed to target the virulence genes of these bacteria and detailed primer sequences are listed in **Table S2** and the performance of each primer set shown in **Figure R2**. The thermal cycling process was performed using CFX Opus 96 Real-Time PCR system (Bio-Rad) with following procedure: initial denaturation at 95 °C for 15 min and 40 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 30 s, and extension at 70 °C for 30 s. The fluorescence signal was detected at every cycle, and the Ct values were obtained. The enhanced detection performance was calculated using the Ct values of each bacterial concentration. First, each Ct value in Table S5 was converted to its reciprocal. Then, each reciprocal was divided by the reciprocal of the Origin for each concentration, and the result was multiplied by 100 to calculate the percentage. Finally, the difference between each Ct value were analysed (**Table S6**).

**Evaluation of Thermo-URCHANO performance using food-poisoning bacteria**

*S. aureus*, *S. enteritidis*, *L. monocytogenes*, and *B. cereus* were cultured in LB broth at 37 °C overnight in a shaking incubator. The CFU value of each bacterial suspension was determined by measuring the optical density at 600 nm (OD 600) using a UV spectrometer and diluting

with fresh LB broth to $10^5$ CFU/mL. The diluted bacterial suspensions were concentrated by centrifugation (RPM 13,500, 1 min) and re-suspended in LB broth (50 μL). Rubber gloves, aprons, eggs, and sausages were purchased from local grocery stores and rinsed with 99% ethanol and D.W. before use. Each bacterial mixture was dropped onto the kitchenware and food samples, which were then left unattended until the liquid evaporated. The area where the bacteria were dropped was gently rubbed using Thermo-URCHANO swabs (0.5× 0.5 cm) to capture the bacteria. The Thermo-URCHANO swabs were then placed in PBS (1 mL) at 37 °C for 10 min to release the captured bacteria. Then, the released bacteria were concentrated, thermally lysed at 95 °C for 5 min, and analyzed using RT-PCR employing the same method as mentioned above.

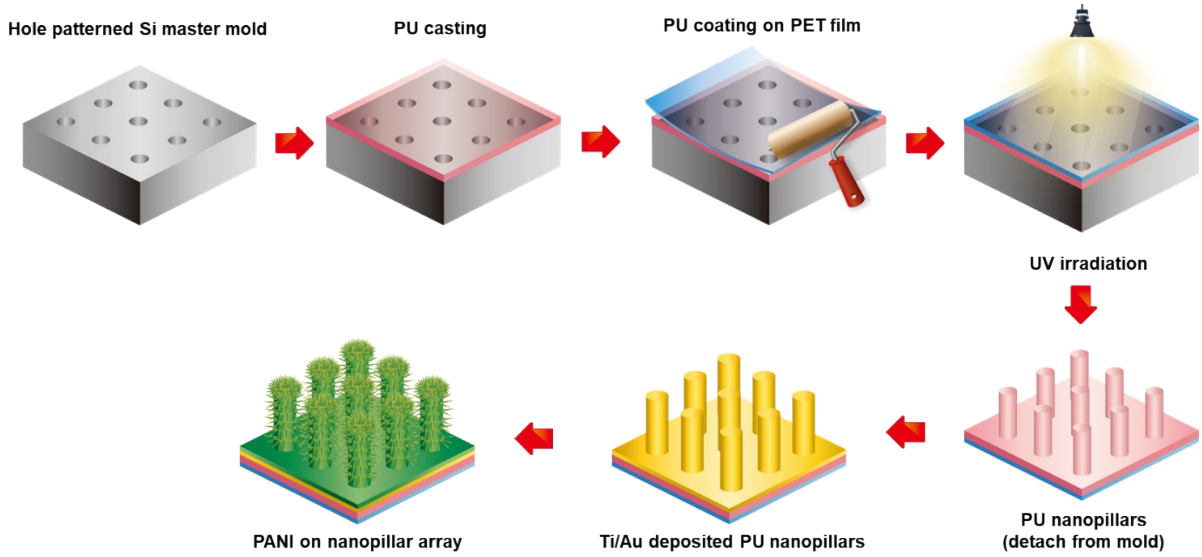


**Figure S1.** Schematic illustration of the fabrication process of URCHANO on a nanopillar array

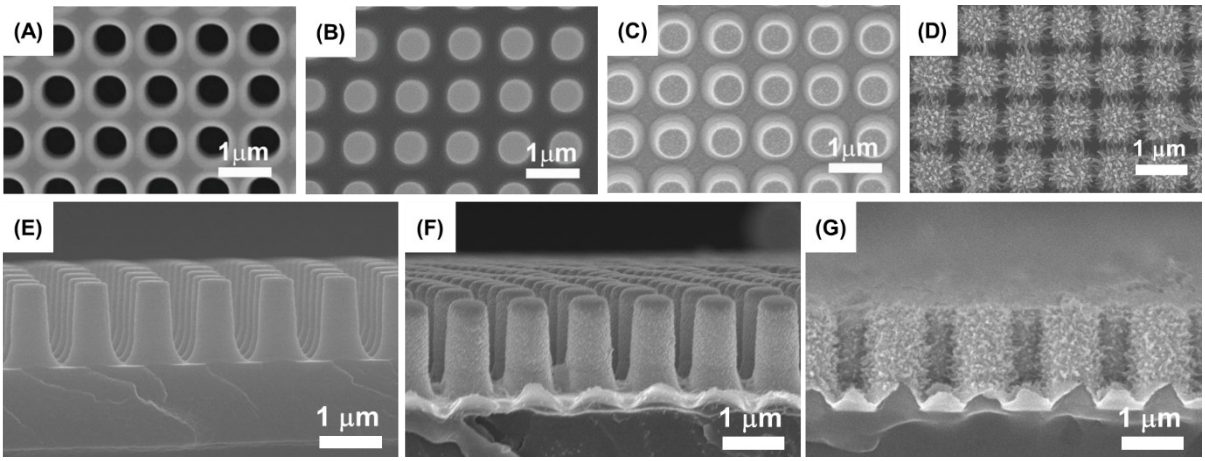


**Figure S2.** Top-view scanning electron microscopy (SEM) images of (A) the nanohole Si master mold, (B) polyurethane (PU) nanopillars, (C) Ti/Au-deposited PU nanopillars (Au substrate), and (D) URCHANO. Side-view SEM images of the (E) PU nanopillars, (F) Au substrates, and (G) URCHANO.

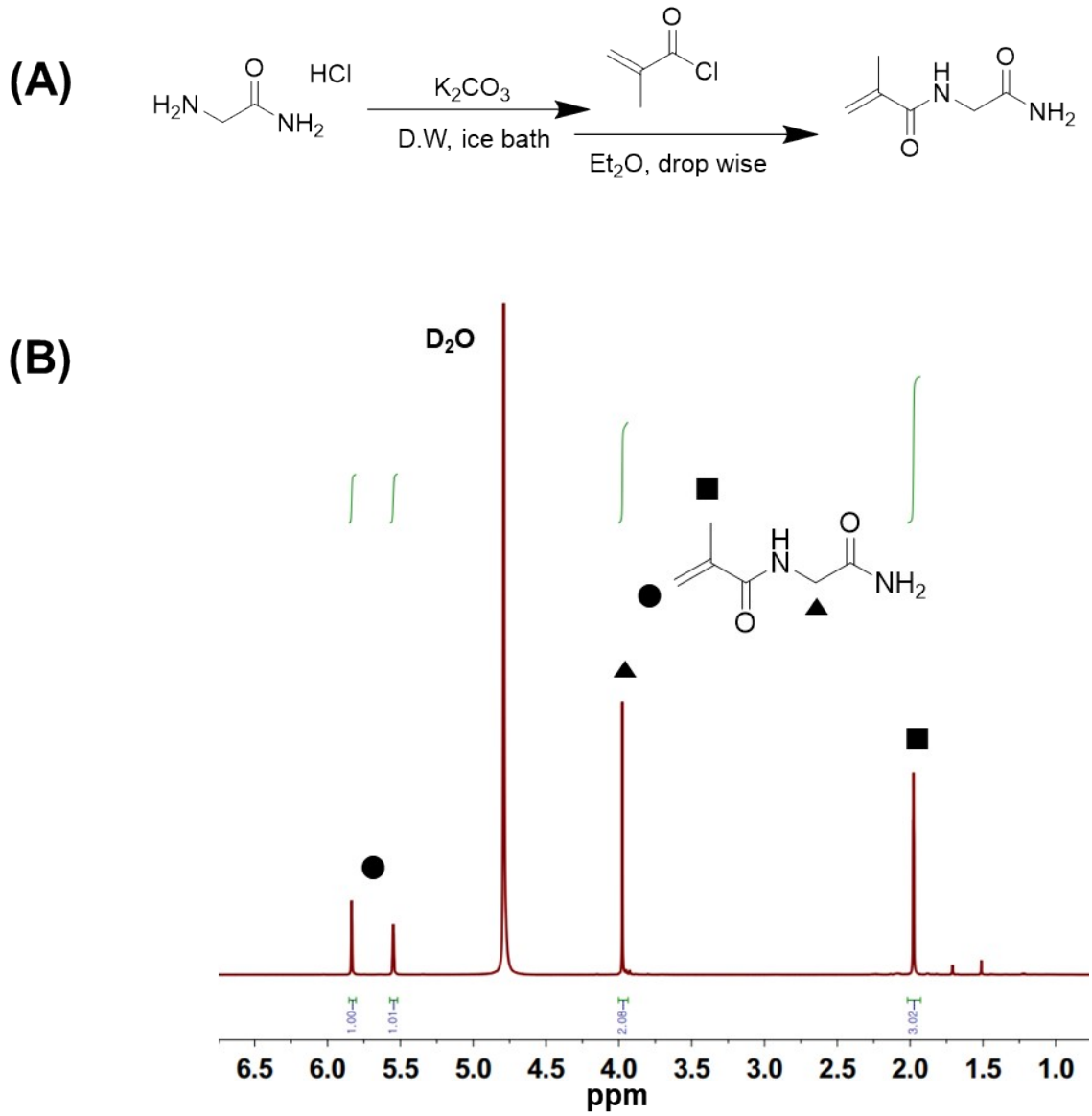


**Figure S3.** Schematic illustration of MNAGA monomer synthesis (a) and 1HNMR spectra of synthesized MNAGA monomer (b). Peaks at 5.85 and 5.59 ppm belong to ethylene moieties(●), 4.0 ppm were aliphatic hydrocarbon (▲) and 2.0 were originated from methyl group(■).

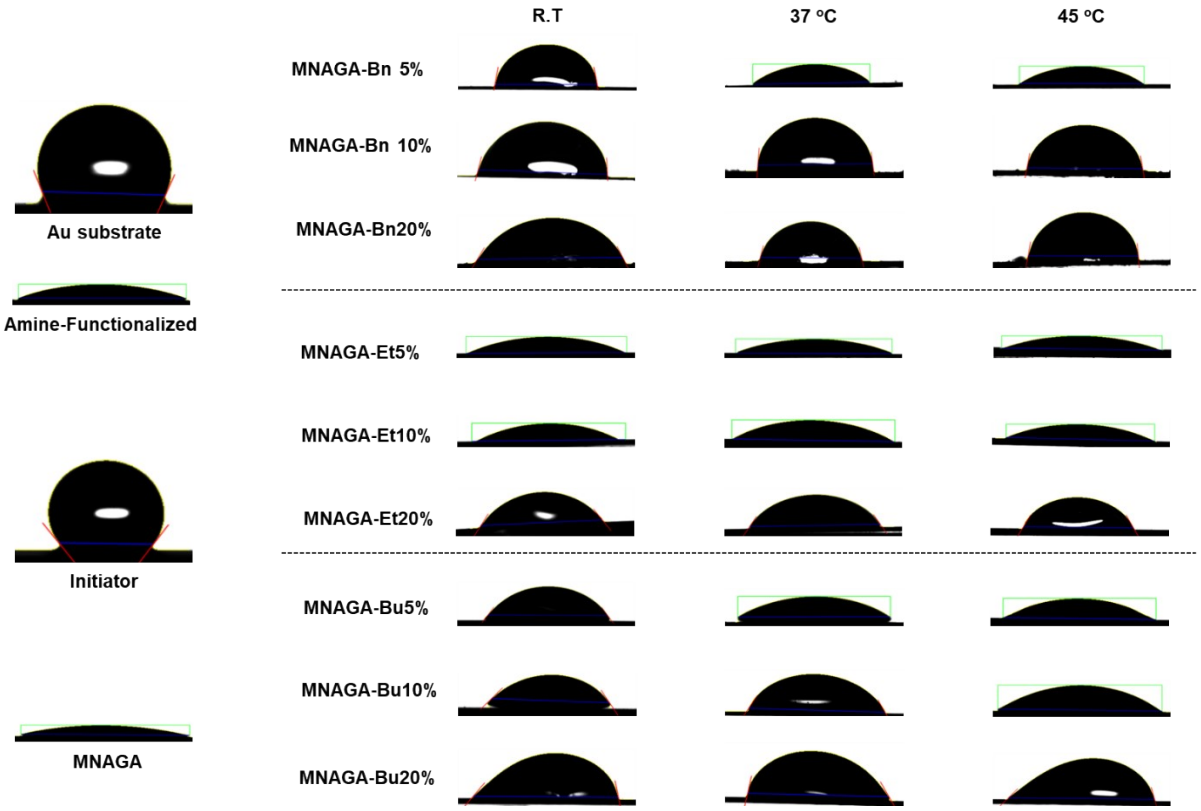


**Figure S4.** Static water contact angles of the Au substrate with various types of modifications at 25 °C, 37 °C, and 45°C

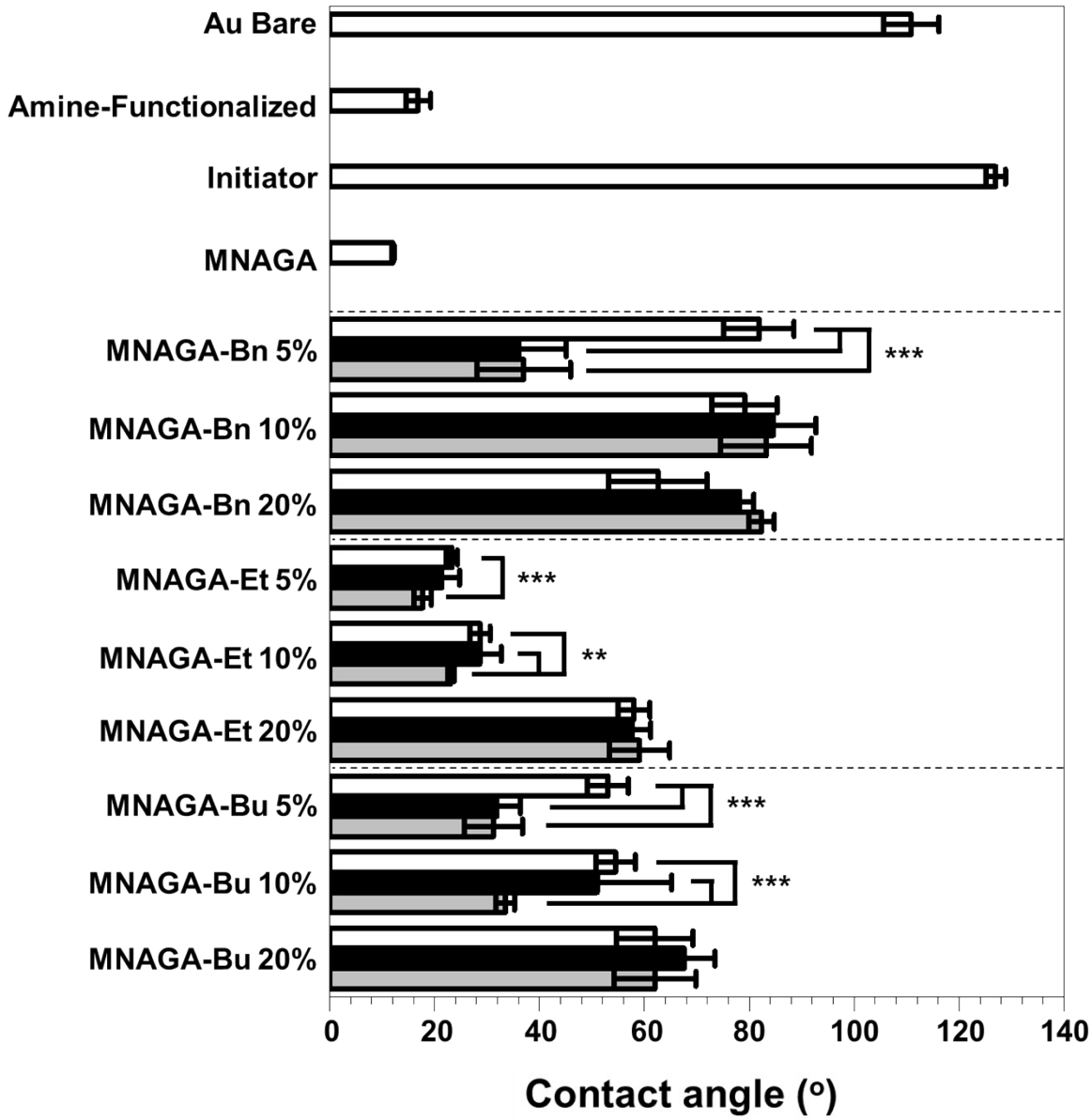


**Figure S5.** Static water contact angles of the Au substrate with various types of modifications at 25 °C, 37 °C, and 45°C. Each value represents the mean of nine measurements from three replicates. The error bars show the 95% confidence limit (white: 25 °C, black: 37 °C, gray: 45 °C, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

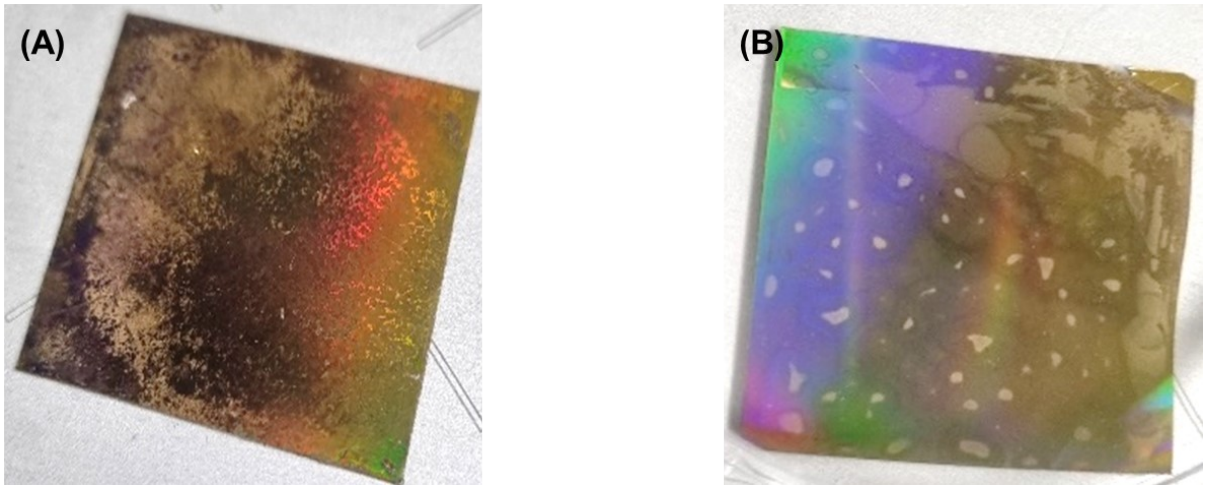


**Figure S6.** Photographs of Au substrates coated with (A) MNAGA-Bn 20% and (B) MNAGA-Bu 20%.

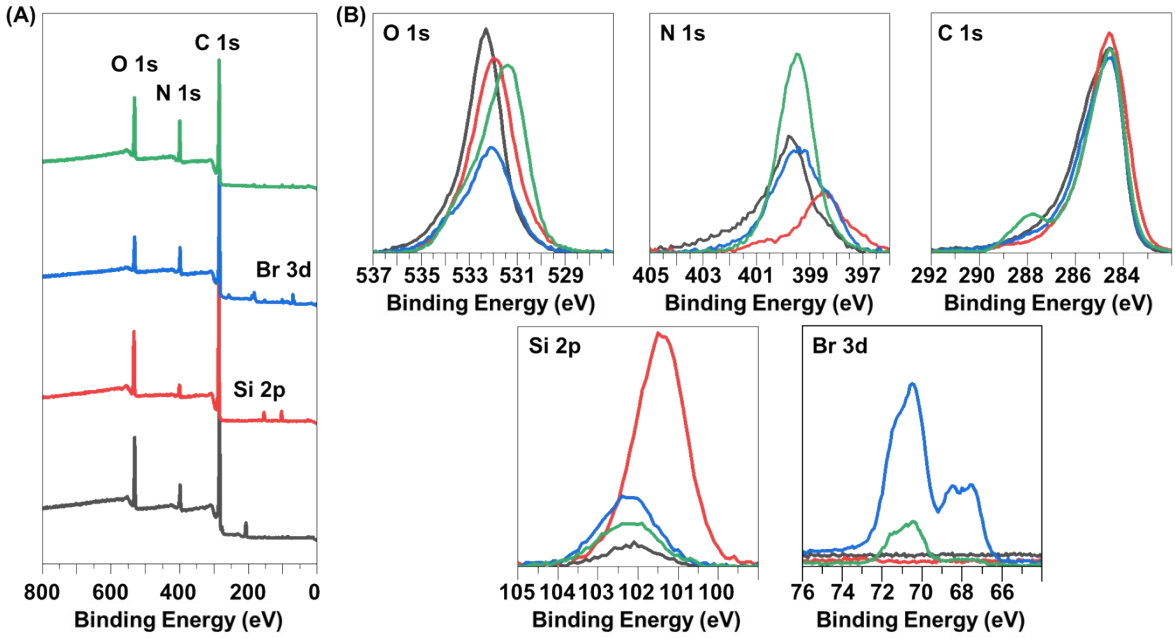


**Figure S7.** XPS spectra of uncoated (black), amine-functionalized (red), BiBB initiator-immobilized (blue), and MNAGA-Bn5%-coated URCHANO (Thermo-URCHANO) (green). Each graph displays the (A) survey scan and (B) high-resolution C 1s, N 1s, O 1s, Si 2p, and Br 3d.

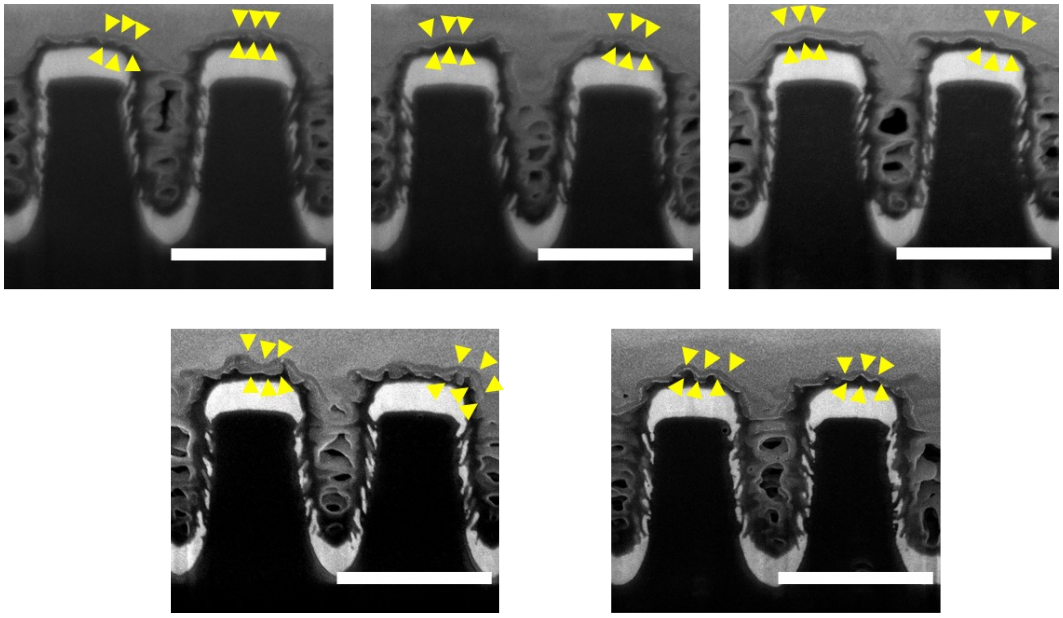

**Figure S8.** FIB-SEM images of Thermo-URCHANO (Scale bar 1 μm). The yellow arrows indicates the MNAGA-Bn5% coating layer.

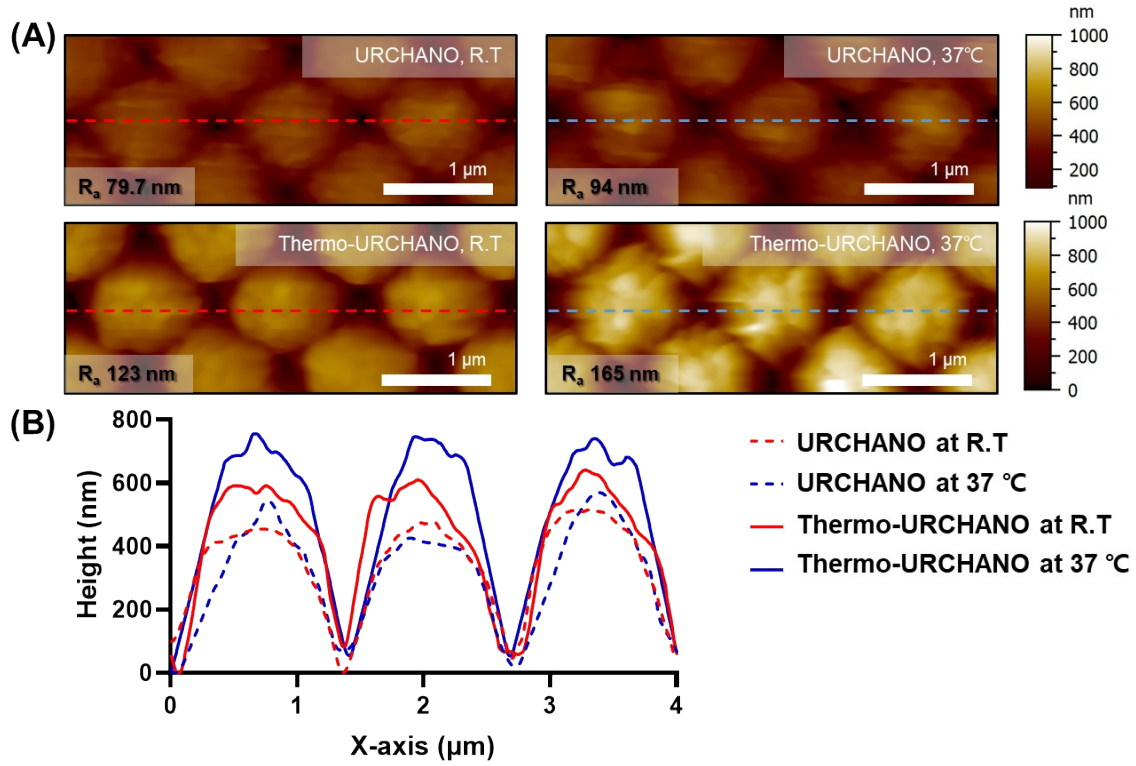


**Figure S9**. **(A)** AFM images of Thermo-URCHANO and uncoated-URCHANO at R.T and 37 °C, and **(B)** cross-sectional profile graph.

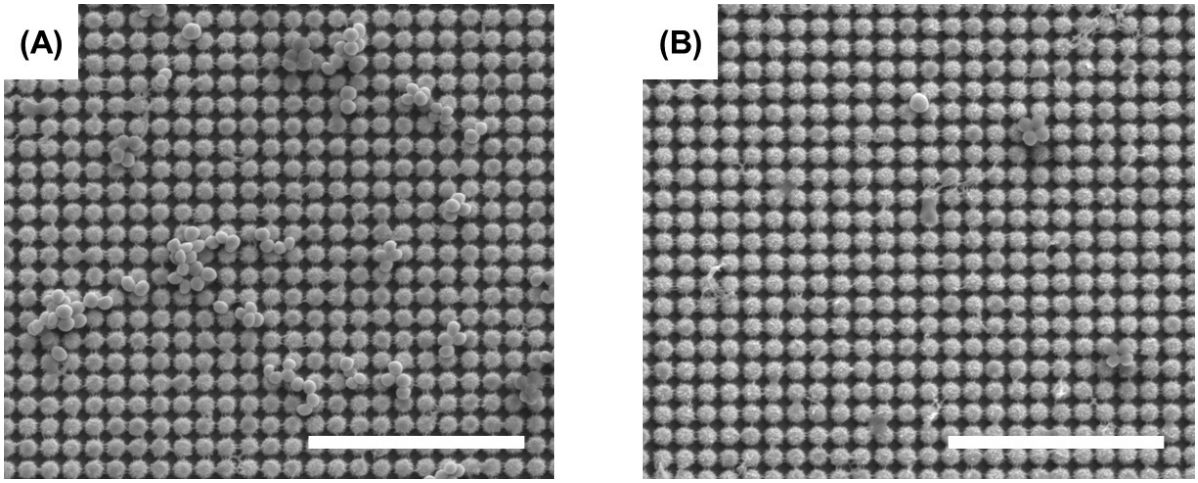


**Figure S10.** SEM images of Thermo-URCHANO after bacterial release at (A) 25 °C and (B) 37 °C. Scale bar: 10 μm.

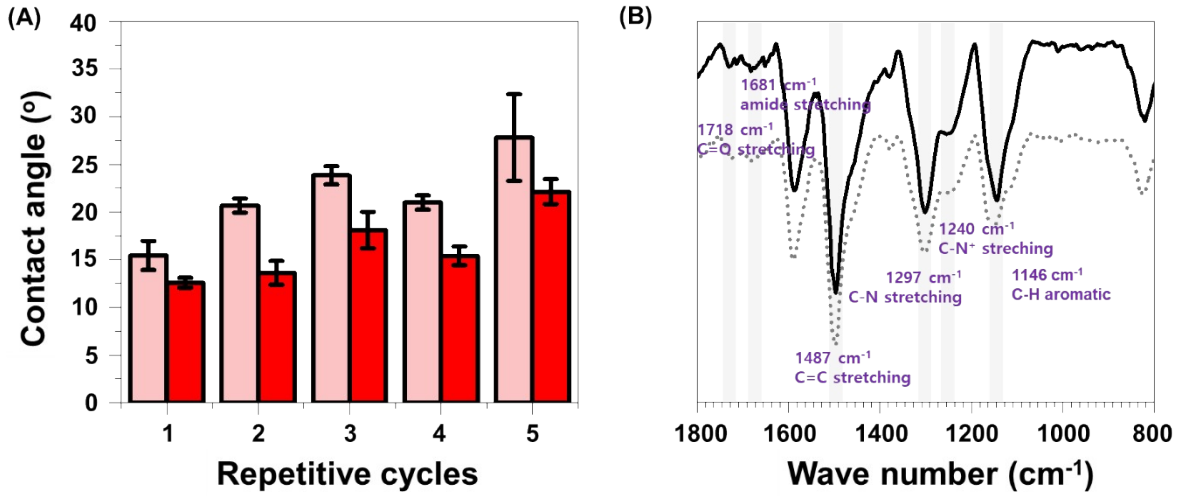


**Figure S11.** (A) Static water contact angles of Thermo-URCHANO with five repetitive cycles from 25 °C (light pink) to 37 °C (red). (B) FTIR spectra of uncoated URCHANO (solid line) and Thermo-URCHANO (dashed line) after five thermal cycles.

| Co-polymer | Feeding ratio (mmole) | | Mass (mg) | |
|---|---|---|---|---|
| | MNAGA | Benzyl acrylate | MNAGA | Benzyl acrylate |
| **MNAGA-Bn 5% -** | 1.425 | 0.075 | 202.45 | 12.16 |
| **MNAGA-Bn 10% -** | 1.35 | 0.15 | 191.79 | 24.33 |
| **MNAGA-Bn 20% -** | 1.2 | 0.3 | 170.48 | 48.66 |
| | MNAGA | Ethyl acrylate | MNAGA | Ethyl acrylate |
| **MNAGA-Et 5% -** | 1.425 | 0.075 | 202.45 | 7.51 |
| **MNAGA-Et 10% -** | 1.35 | 0.15 | 191.79 | 15.02 |
| **MNAGA-Et 20% -** | 1.2 | 0.3 | 170.48 | 30.03 |
| | MNAGA | Butyl acrylate | MNAGA | Butyl acrylate |
| **MNAGA-Bu 5% -** | 1.425 | 0.075 | 202.45 | 9.61 |
| **MNAGA-Bu 10% -** | 1.35 | 0.15 | 191.79 | 19.23 |
| **MNAGA-Bu 20% -** | 1.2 | 0.3 | 170.48 | 38.45 |

**Table S1.** Table of feeding ratio and mass for each copolymer coating

| Target bacteria | Target genes | Forward primers (5′→3′) | Reverse primers (5′→3′) |
|---|---|---|---|
| *Salmonella enteritidis* | invA | GCCGGAAGTATTGTTACGCG | CAACACATAGCCAAGCTCCC |
| *Staphylococcus aureus* | Hla | AAACACGTATAGTCAGCTCAGTAACAAC | CCTGTTTTTACTGTAGTATTGCTTCCA |
| *Listeria monocytogenes* | actA | GACTTGCTTTGCCAGAGACG | CTTCTGTTGGGATTGGTGGG |
| *Bacillus cereus* | EntFM | ACATACAACGCTGTAATCGG | GCGATTGAAGATGTATCTCC |

**Table S2.** Target bacteria, target genes, and primer sequences used in this study.

| | 25°C | 37°C | 45°C |
|---|---|---|---|
| **Au substrate** | 110.9 ± 5.3° | - | - |
| **Amine-functionalized-** | 16.9 ± 2.4° | - | - |
| **BiBB Initiator immobilized-** | 127.0 ± 1.8° | - | - |
| **MNAGA-** | 12.0 ± 0.2° | - | - |
| **MNAGA-Bn 5% -** | 81.8 ± 6.9° | 36.1 ± 9.0° | 37.0 ± 8.9° |
| **MNAGA-Bn 10% -** | 79.1 ± 6.2° | 84.6 ± 8.1° | 83.2 ± 8.7° |
| **MNAGA-Bn 20% -** | 62.6 ± 9.3° | 78.2 ± 2.7° | 82.3 ± 2.5° |
| **MNAGA-Et 5% -** | 23.3 ± 1.03° | 21.4 ± 3.4° | 17.7 ± 1.6° |
| **MNAGA-Et 10% -** | 28.7 ± 1.9° | 28.6 ± 4.1° | 23.1 ± 0.6° |
| **MNAGA-Et 20% -** | 58.0 ± 3.0° | 57.6 ± 3.6° | 59.1 ± 5.7° |
| **MNAGA-Bu 5% -** | 53.0 ± 3.9° | 31.9 ± 4.4° | 31.2 ± 5.6° |
| **MNAGA-Bu 10% -** | 54.5 ± 3.8° | 51.0 ± 14.2° | 33.5 ± 1.8° |
| **MNAGA-Bu 20% -** | 62.0 ± 7.3° | 67.6 ± 5.9° | 62.0 ± 7.8° |

**Table S3.** Static water contact angles of the Au substrates with various types of modifications at 25°C, 37°C, and 45°C. Each value represents the mean of nine measurements from three replicates, and the standard deviation (±) value represents the 95% confidence limit.

| | C 1s | N 1s | O 1s | Si 2p | Br 3d |
|---|---|---|---|---|---|
| **URCHANO** | 74.2 | 10.09 | 15.24 | 0.47 | - |
| **Amine functionalized-URCHANO** | 73.74 | 5.57 | 15.85 | 4.84 | - |
| **BiBB initiator immobilized-URCHANO** | 75.74 | 10.86 | 10.19 | 1.76 | 1.46 |
| **Thermo-URCHANO** | 69.83 | 12.88 | 15.98 | 1.06 | 0.25 |

**Table S4.** Atomic percentages of uncoated, amine-functionalized URCHANO, BiBB initiator-immobilized URCHANO, and Thermo-URCHANO.

| CFU/mL | Thermo-URCHANO | | Uncoated URCHANO | | Origin |
|---|---|---|---|---|---|
| | 25 °C | 37 °C | 25 °C | 37 °C | |
| $10^5$ | 28.8 ± 1.2 | 23.8 ± 0.3 | 27.8 ± 1.1 | 26.1 ± 2.2 | 22.8 ± 0.5 |
| $10^4$ | 33.5 ± 1.8 | 29.5 ± 2.1 | 33.6 ± 2.1 | 33.7 ± 2.2 | 28.7 ± 0.7 |
| $10^3$ | 37.9 ± 1.6 | 33.7 ± 0.8 | 38.7 ± 1.9 | 39.6 ± 2.1 | 32.1 ± 0.6 |
| $10^2$ | 40.1 ± 2.1 | 38.6 ± 1.4 | 40.2 ± 1.8 | 40.6 ± 2.0 | 36.3 ± 1.2 |

**Table S5.** Ct values of *S. aureus* at different concentrations captured by Thermo-URCHANO and uncoated URCHANO and released with and without thermal treatment. Each value represents the mean of 18 measurements from six replicates.

| CFU/mL | Thermo-URCHANO | | Uncoated URCHANO | | Origin |
|---|---|---|---|---|---|
| | 25 °C | 37 °C | 25 °C | 37 °C | |
| $10^5$ | 79.2 | 95.8 | 82.0 | 87.4 | 100 |
| $10^4$ | 85.7 | 97.3 | 85.4 | 85.2 | 100 |
| $10^3$ | 84.6 | 95.3 | 82.9 | 81.1 | 100 |

**Table S6.** Values expressed as percentages by dividing each reciprocal of Ct values by the reciprocal of the Origin.

Reference

1. K. H. Kim, M. Yang, Y. Song, C. H. Kim, Y. M. Jung, N.-H. Bae, S.-J. Chang, S. J. Lee, Y. T. Kim, B. G. Choi and K. G. Lee, *Nano Converg.*, 2021, **8**, 30

2. Y. Ren, Y. Zhang, W. Sun, F. Gao, W. Fu, P. Wu and W. Liu, *Polymer*, 2017, **126**, 1–8.

3. X. Xue, L. Thiagarajan, S. Braim, B. R. Saunders, K. M. Shakesheff and C. Alexander, *J. Mater. Chem. B*, 2017, **5**, 4926–4933.