Electronic Supplementary Information (ESI)

A Mild Deposition of Metallic Materials on a Plastic Film

Enabled by Phage Display Peptides

Swathi Swaminathan,³ and Yue Cui^{1,2*}

¹Department of Electrical Engineering and Computing Systems, University of Cincinnati, Cincinnati, OH 45221

²Department of Mechanical and Materials Engineering, University of Cincinnati, Cincinnati, OH 45221

³Department of Biological Engineering, Utah State University, Logan, Utah 84322, USA

* Corresponding author, E-mail: <u>cuiy3@ucmail.uc.edu</u>

PHAGE DISPLAYED SCREENING

Materials

LB Medium: Per liter: 10 g Bacto-Tryptone, 5 g yeast extract, 5 g NaCl. Autoclave, store at room temperature. IPTG/Xgal Stock: Mix 1.25 g IPTG (isopropyl-β-D-thiogalactoside) and 1 g Xgal (5-Bromo-4- chloro-3-indolyl-β-D-galactoside) in 25 ml DMF (dimethyl formamide). **LB/IPTG/Xgal Plates**: 1 liter LB medium + 15 g/l agar. Autoclave, cool to < 70°C, add 1 ml IPTG/Xgal Stock per liter and pour. Store plates at 4°C in the dark. **Top Agar**: Per liter: 10 g Bacto-Tryptone,7 g eletrophoresis grade agarose. **Tetracycline Stock (suspension)**: 20 mg/ml in 1:1 Ethanol:Water. Store at –20°C. Vortex before using. LB+Tet Plates: LB medium + 15 g/l Agar. **Blocking Buffer:** 0.1 M NaHCO3 (pH 8.6), 5 mg/ml BSA, 0.02% NaN3 (optional). **TBS**: 50 mM Tris-HCl (pH 7.5), 150 mM NaCl. Autoclave, store at room temperature. **PEG/NaCl:** 20% (w/v) polyethylene glycol–8000, 2.5 M NaCl. **Iodide Buffer:** 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 4 M sodium iodide (NaI).

E.Coli Strain

The recommended E. coli host strain ER2738 (F' proA+ B+ lacIq Δ (lacZ) M15 zzf::Tn10(TetR)/fhuA2 glnV Δ (lac-proAB) thi-1 Δ (hsdS-mcrB)5. [rk – mk – McrBC–]) is a robust F+/ recA+ strain with a rapid growth rate and is well-suited for M13 propagation and does not produce spontaneous in vivo recombination events with M13. This already provided in the phage display kit.

Biopanning Procedures

Day One: 1. 10 μ l of the phage solution is added to the substrate contained in a P60 with 1.5ml 0.1%TBS-Tween 20 and incubated for 1 hour. Then the substrate is washed with 0.1% TBST for 10 times and 15 mins each. The bound phage is then eluted 1.5 ml of 0.2 M Glycine-HCl (pH 2.2), 1 mg/ ml BSA into a microcentrifuge tube and neutralized with 250 μ l 1 M Tris-HCl, pH 9.1. The pH of the resulting eluent is 7.5, which is tested with a pH strip. Amplify 1.5ml of this unamplified eluate to the 20-ml ER2738 overnight culture and incubated with vigorous shaking for 4.5 hours at 37°C. Note: The remaining eluate was stored overnight at 4°C . After the amplification process, the culture was transferred to a centrifuge tube and 1/5 volume of 20% PEG/2.5 M NaCl was added to it. The phage was allowed to precipitate overnight at 4°C .

Day Two: The PEG precipitation was centrifuged at 12,000 g for 15 minutes at 4°C. The supernatant was suspended in 1 ml of TBS.The mixture was transferred to a fresh microcentrifuge tube and reprecipitated by adding 1/5 volume of 20% PEG/2.5 M NaCl. This was incubated at 4 C for 2 hours. After precipitation, the phage was collected in 200 μ l of TBS after centrifuging at 14,000 rpm for 10 minutes at 4°C. This was the amplified eluate. From Round 1. Round 2 was repeated by adding 10 μ l of Round 1 amplified eluate to 1.5ml of 0.3% TBST and the entire process was repeated as above.

Day Three: The third round of panning was conducted by adding 10µl of Round 2 amplified eluate to 1.5ml of 0.3% TBST and the entire process was repeated as round 1 panning. The unamplified eluate was tittered upto the fifth dilution on LB/IPTG/Xgal plates. Plaques from this was used for sequencing.

Purification of Plaques for Sequencing

The plaque amplification was conducted as described in round 1 panning procedure. After centrifugation, 500 μ l of the phage-containing supernatant was transferred to a fresh microfuge tube. 200 μ l of 20% PEG/2.5 M NaCl was added to this mixture and incubated in room temperature for 20 min. This was followed by centrifugation at 14,000 rpm for 10 minutes at 4°C and the supernatant was discarded. The pellet was suspended in 100 μ l of Iodide Buffer by vigorously tapping the tube. This was followed by the addition of 250 μ l of ethanol and incubated for 20 mins at room temperature. Then, centrifugation was conducted at 14,000 rpm for 10 minutes at 4°C, and the supernatant was discarded. The pellet was discarded at 14,000 rpm for 10 minutes at 4°C, and the supernatant was discarded. The pellet was washed with 0.5 ml of 70% ethanol ,re-spun, supernatant discarded, and the pellet was dried under vacuum for 4 hours. After drying, the pellet was suspended in 50 μ l of TE buffer. 25 μ l of the plaque DNA was sent to the sequencing company to identify the phage displayed peptides.

PHOTOLITHOGRAPHY ON PET

A 3x5 cm PET film was fixed on glass to conduct photolithography using AZ 5214. The films were coated with HMDS at 4000 rpm and 45 s, followed by AZ 5214 at 2000 rpm and 30 s. The films were soft baked at 105 °C for 1 min and covered with a photomask. Exposure was done at 6 s using a UV source of 20 mJ/Wcm⁻². The films were finally developed for 1 min 30 secs for further fluorescent characterization.

FLUORESCENT CHARACTERIZATION ON PLAIN PET OR PATTERNED PET

A plain PET or patterned PET film was exposed to 10 μ l of PET binding phage displayed peptides in TBS buffer for 0.5 h in a 35mm petri dish, followed by washing with TBS buffer, and incubated with 0.1 M NaHCO₃, 1% BSA, at pH 8.6 for 0.5 h in order to reduce the non-specific adsorptions of antibody and FITC on the substrate. Then, the surface was exposed to anti-M13

phage antibody (1 μ g in 1.5 ml buffer) for 0.5 h with gently shaking, and then rinsed with TBS to remove the unconjugated antibody. Finally, 10 μ l of avidin–FITC label (2.0 unit ml⁻¹) was applied to the biotin conjugated phage through a biotin–avidin interaction, and the surface was exposed to the FITC label in 1.5 ml of TBS for 0.5 h, and then rinsed several times with TBS to remove the unconjugated avidin–FITC label. A control experiment was also conducted with M13 phage without phage displayed peptides, following the above Experimental procedures. The color intensity of the surface was observed by a fluorescence microscope.

BI-FUNCTIONAL PEPTIDE SYNTHESIS OF SILVER NANOPARTICLE ON PET

A 1cm x 1cm PET film (both patterned and non-patterned) was incubated with 10 μ l of 50 mM bifuctional peptide (DEYCCNN-GGGGG-NPSSLFRYLPSD), followed by 1.5 ml of 30 mM Silver nitrate solution in PBS buffer of pH 7 for 3 days in a 35 mm petri dish. After incubation, the substrate was washed by PBS buffer at pH 7.5 and water, followed by blow drying. This enabled the formation of silver nanoparticles on the plain PET film. The silver nanoparticles were characterized with an optical microscope and a SEM.