Supplementary Material for Chemical Communications

Supplementary Information

Materials and Methods

Materials. DL-β-Hydroxybutyryl coenzyme A (Sigma, St. Louis, MO), N,N-Bis(carboxymethyl)-L-lysine-Hydrate (Fluka, Milwaukee, WI) and (3-Glycidoxypropyl) trimethoxysilane (Gelest Inc, Tullytown, PA) were used as received. Ni-NTA agarose (Ni-NTA coupled to a Sepharose CL-6B support) and Nile Red (Nile Blue A Oxazone) were obtained from Qiagen (Valencia, CA) and Sigma (St. Louis, MO), respectively.

Preparation of PHA synthase. PHA synthase was prepared as described previously.[1] $PhaC_{Re}$ encoding PHA synthase was isolated from *Ralstonia eutropha* H16 (DSM428), cloned into pET19-b (Novagen, Madison, WI), and then introduced into *Escherichia coli* BL21 pLysS (Novagen) for protein overexpression. The enzyme was purified using Ni-NTA agarose resin under native conditions according to the manufacturer's instructions (Qiagen, Valencia, CA). Protein concentration was determined by the Bradford method with bovine serum albumin (BSA) as a standard [2].

***In vitro* synthesis of PHB.** PHB (with His-tagged enzyme covalently attached to one end of the polymer chain) was prepared by *in vitro* synthesis in 1 mL of 20 m*M* potassium phosphate buffer (pH 7.0) containing 200 m*M* NaCl, 5 m*M* 3-hydroxybutyryl-CoA and 0.5 μ*M* PHA synthase at room temperature. The polymerization reaction was followed spectrophotometrically by measuring the free thiols released during polymerization (Ellman's assay).[3] A 10 μl aliquot of the reaction mixture was taken at each time point and mixed with 390 μl of 5 m*M* sodium acetate buffer (pH 4.7) containing 50 m*M* NaCl and 0.5 m*M* EDTA. 690 μl of 40 m*M*

sodium phosphate buffer (pH 7.6) containing 2 m*M* EDTA and 10 μl of 100 m*M* 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were subsequently added and mixed at room temperature for 2 min. Absorbance of the solution was measured at 412 nm using dH_2O as a blank. The molar extinction coefficient ($cm^{-1}M^{-1}$) of DTNB in reacting with free thiols is 13,600.[3] After 1 hr polymerization, PHB granules were spun down at 14,000 x *g* for 5 min. The pellet was rinsed with methyl alcohol and then dissolved in chloroform.

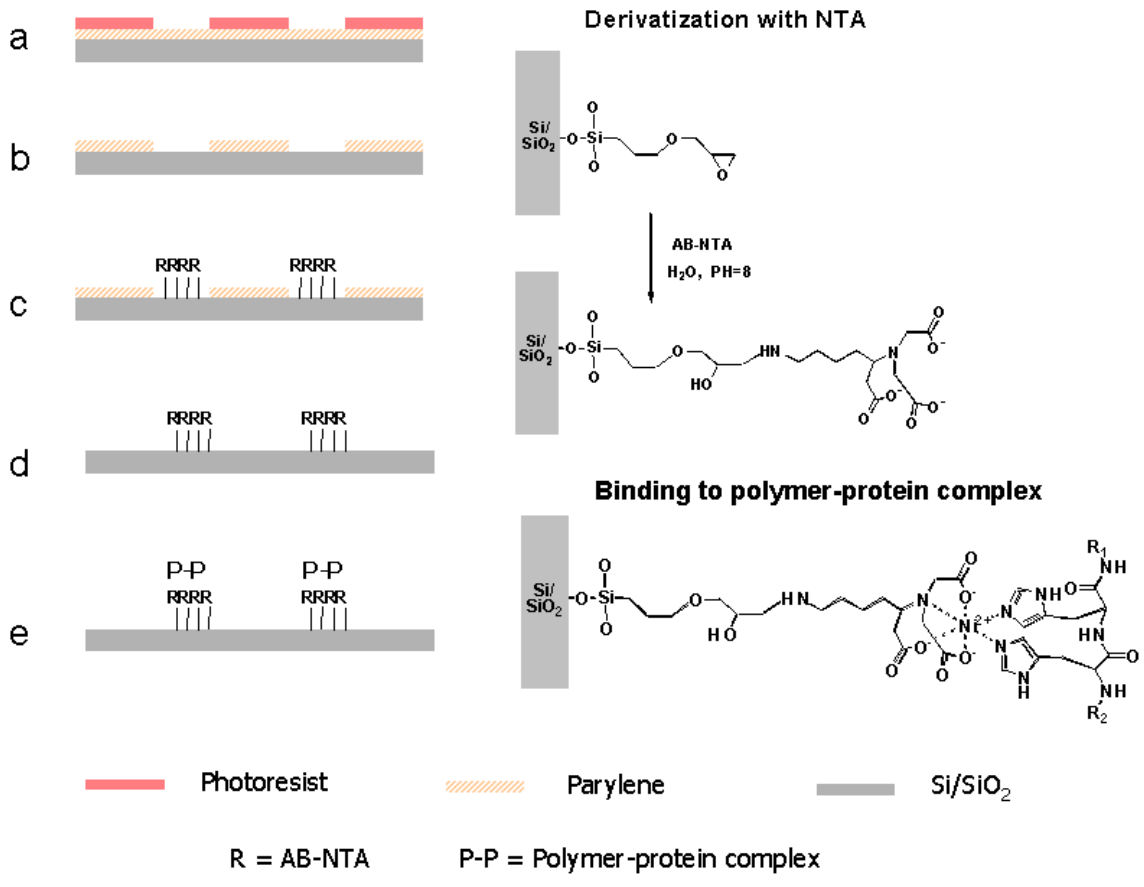
Derivatization of patterned silicon with Ni-NTA. A silicon wafer was initially patterned with a 1 μm-thick Parylene film through conventional photolithography and etching techniques.[4] 3-inch (111) prime grade silicon wafers were cleaned in piranha solution (3 H_2SO_4 : 1 H_2O_2) for 10 min, and then rinsed with deionized water and blown dry with high purity nitrogen gas. Parylene C dimmer (di-para-xylylene) was vapor-deposited on the silicon wafer with a PDS-2010 Labcoater 2 Parylene deposition system (Specialty Coating Systems, Indianapolis, IN). The parylene coated wafer was primed with hexamethyldisilazane (HMDS) to achieve optimum adhesion between the parylene and the photoresist film. OGC 897-12i positive photoresist was spun on the wafer at 4,000 rpm for 30 sec, followed by pre-baking on a hotplate at 90^{o}C for 5 min. The photoresist was exposed with UV light through a photomask using a GCA 6300 DSW 10x i-line (365 nm) stepper, and then developed in a CD-26 developer solution for 90 sec. The exposed region of the parylene film was etched using a PlasmaTherm 72 RIE System. The residual photoresist was removed by rinsing with acetone, isopropyl alcohol and then deionized water. The exposed Si/SiO_2 region was subsequently derivatized with Ni-NTA according to the method reported by Adachi *et al*.[5] The parylene-patterned silicon wafer was oxygen (O_2) plasma cleaned, immersed in a 95% ethanolic solution containing 2% (vol/vol) (3- glycidyloxypropyl) trimethoxysilane for 3 hrs at 60^{o}C and washed with water. Next, the wafer was incubated in 0.1 *M* sodium

phosphate buffer (pH 8.0) containing 10 m*M* N,N- Bis(carboxymethyl)-L-lysine hydrate (AB-NTA) for 4 hrs at 60^{o}C. The wafer was washed with deionized water and the Parylene was peeled off the surface. The wafer was then incubated in 5 m*M* glycine buffer (pH 8.0) containing 10 m*M* $NiCl_2$ for 2 hrs at room temperature to charge the surface-immobilized NTA groups with Ni ions. The wafer was rinsed with water and stored in water until use.

Graft-onto reaction of $(His)_{10}$-tag PHA synthase-PHA polymer complex on Ni-NTA agarose beads and silicon surfaces. The aqueous buffer used for storing Ni-NTA agarose beads was exchanged with methyl alcohol and then with chloroform. A 100 μL-aliquot of the sludge was transferred to 0.5 mL PHB solution (1mg PHB in 1 mL chloroform), and incubated at room temperature for 1 hr with mild agitation. The beads were thoroughly washed with chloroform to remove nonspecifically bound residual polymers from the surface. The beads were dried in air for further characterization. A piece of the Ni-NTA-derivatized silicon wafer was immersed in chloroform containing *in vitro*-synthesized PHB (1mg PHB in 1 mL chloroform), and incubated at room temperature for 1 hr with light agitation. The wafer was then washed thoroughly with chloroform, and blown dry with high purity nitrogen gas. For the immobilization of His-tagged proteins onto the Ni-NTA derivatized silicon wafer, the Ni-NTA silicon wafer was incubated in 50 m*M* Tris buffer (pH 8.0) containing 1 μ*M* enzyme for 30 min at room temperature. The wafer was washed thoroughly and kept in 50 m*M* Tris buffer (pH 8.0) at 4^{o}C until ready for further characterization.

Analysis of the enzyme and polymer grafted onto the surface. PHA synthase immobilized on the silicon wafers was visualized by fluorescence microscopy using a FITC-conjugated antibody. Specifically, the immobilized enzyme was first reacted with an unlabeled rabbit primary antibody raised against *R. eutropha* H16 PHA

synthase, and then with a FITC-conjugated secondary anti-rabbit IgG (Sigma). The grafted PHB film was stained with Nile red (0.5 mg Nile red/ml in DMSO) at 50°C for 5 min, and visualized with fluorescence microscopy. The stained PHB films synthesized on the surface of the agarose beads and the silicon wafer were visualized with fluorescence microscopy using an excitation wavelenth set at 546 ± 10 nm. Figure 1. Process flow schematic of the fabrication steps carried out to produce a patterned silicon wafer with polymer-protein complexes immobilized on the surface. (a) Photoresist patterning on a Parylene film using optical lithography. (b) Reactive ion etching (RIE) of Parylene, followed by removal of the residual photoresist. (c) Derivatization of the exposed Si/SiO2 surface with Ni-NTA. The detailed scheme is shown on the righthand side of the figure. (d) Peeling off the Parylene and (e) grafting of the polymer-protein complex onto the Ni-NTA surface. The binding mechanism of the His-tag to Ni-NTA (transition metal complex) is shown on the right.



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