Electronic Supplementary Material (ESI) for Analytical Methods

Supporting Information

Parallel assay of inkjet printed cytochrome P450

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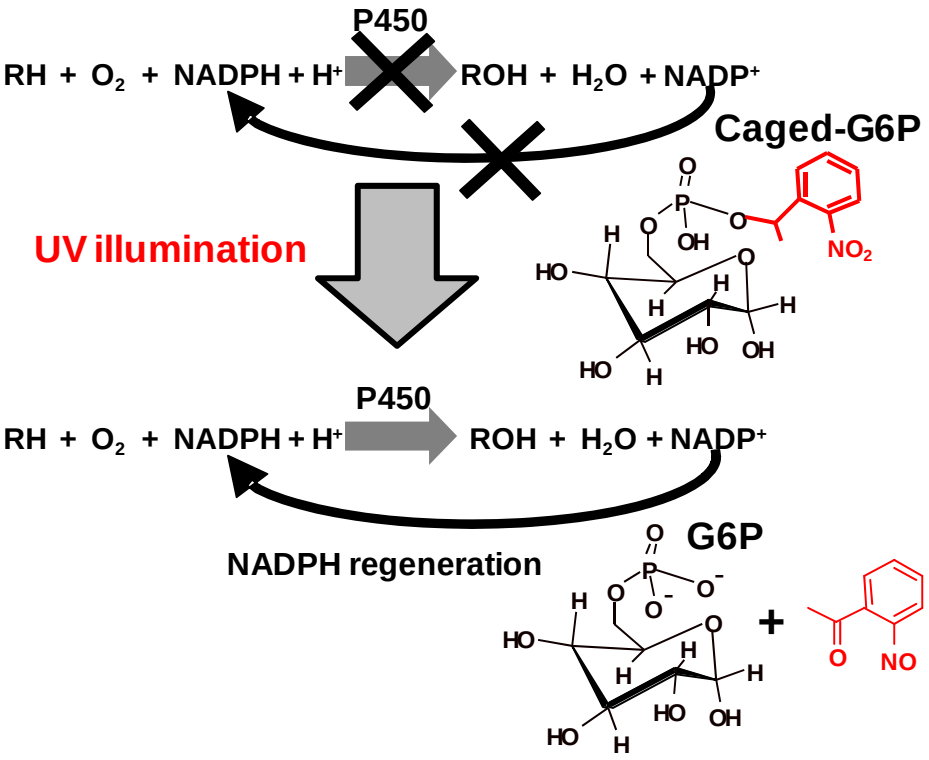

Figure S1: Reaction scheme of the photo-regulated P450 catalysis using caged-G6P.

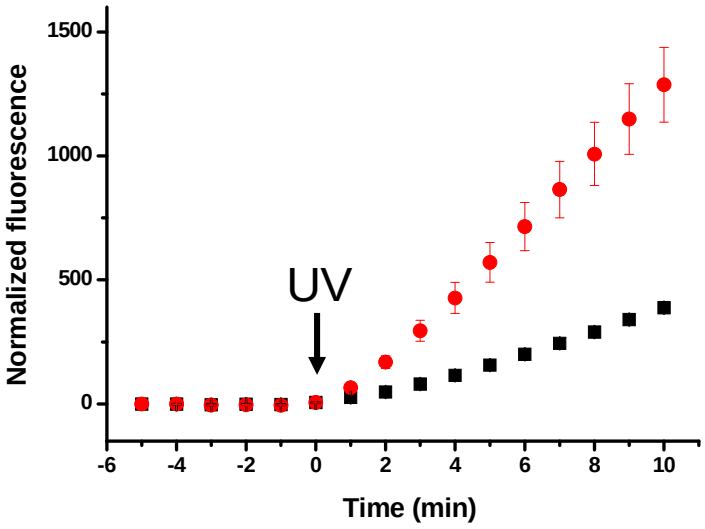

Figure S2: Effect of trehalose on the activity of printed CYP1A1: CYP1A1 was printed with or without trehalose, and the enzymatic activities toward 7-ER (1.5 μM) were compared. Black square: no trehalose, Red circle: 0.01% (w/v) trehalose.

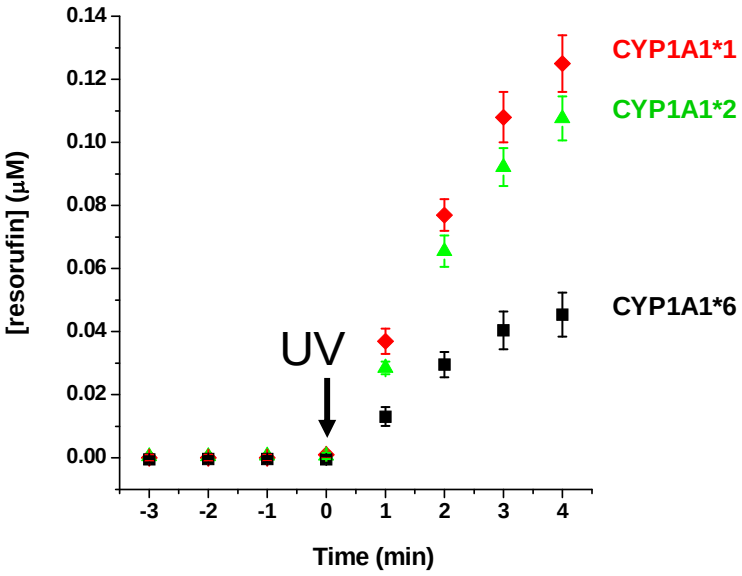

Figure S3: Activities of CYP1A1 variants: Membrane fragments containing one of the CYP1A1 variants (CYP1A1*1 (wild type), CYP1A1*2, CYP1A1*6) were encapsulated into microwells made of PDMS, and their activities were assayed. 7-ER (1.5 μM) was used as the substrate, and the initiation of the P450 catalysis was controlled by using caged-G6P. The activities of P450s were normalized with the concentration of P450. Error bars represent standard deviation from 12 microwells for each type of P450.

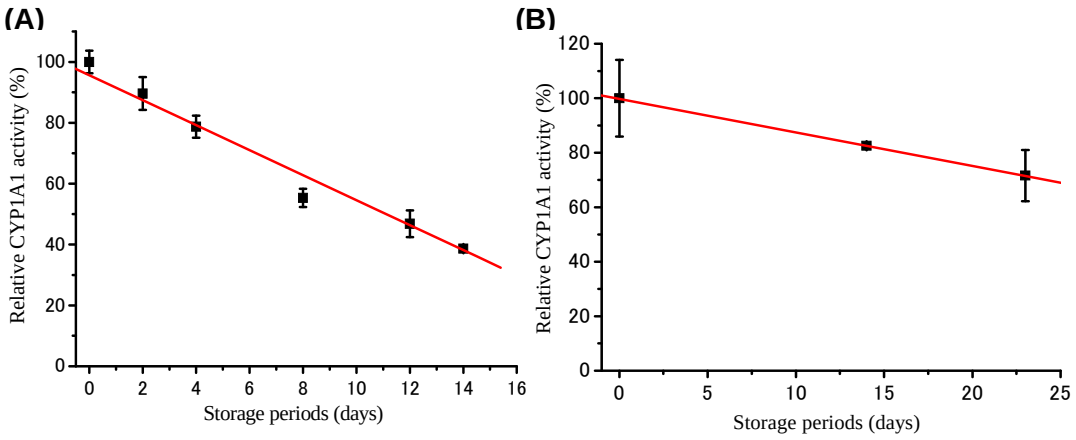

Figure S4: (A) Stability of printed P450 at 4 °C: CYP1A1 was printed into microwells and stored at 4 °C until use. The activity of CYP1A1 toward 7-ER was assayed using G6P. Error bars represent standard deviation from 24 microwells (2 chips).
(B) Stability of P450 in suspended membrane fragments at 4 °C: CYP1A1 in a test tube was stored in the refrigerator at 4 °C until use. (The volume of the solution was ca. 10 µl.) After the incubation for a defined period, residual activities of P450 were measured with 7-ER. The reaction solution contained 0.1M KPB, 0.4 U/ml G6P dehydrogenase, 3 mM magnesium chloride, 1 mM DTT, 0.3 mM G6P, and 0.01 mM $NADP^+$ (total volume: 500 µl). After incubating for 30 min at room temperature, the reaction was terminated by adding 25 µl of 30% trichloroacetic acid. 500 µl chloroform was added to the reaction solution and vigorously mixed for 1 min to extract the reaction product (resorufin). The organic and aqueous phases were separated by centrifugation (2,000g for 1 min) and 250 µl chloroform was transferred to another tube. Then, 500 µl of aqueous solution containing 5 mM NaOH/ 50 mM NaCl was added, and the mixture was vigorously mixed for 1 min. The organic and aqueous phases were separated by centrifugation, and 300 µl of the aqueous phase was transferred to a quartz cell (F20-SQ-5, GL Sciences, Japan) for the fluorescence measurement (F4500, Hitachi, Japan; excitation:530 nm, emission: 583 nm).

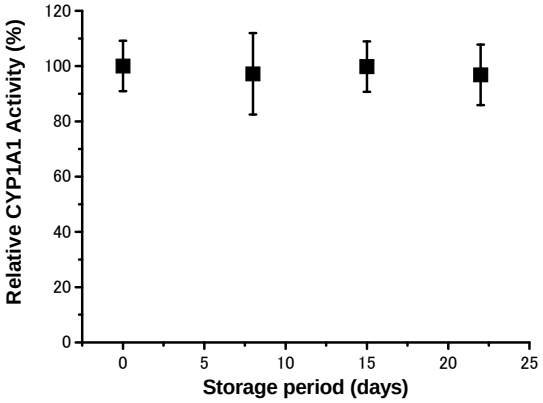

Figure S5: Storage of printed P450 in microwells: Printed CYP1A1 in microwells was stored at -80 °C. After a defined period, the chip was thawed and the residual activity of CYP1A1 toward 7-ER (1.5 μM) was assayed. No freezing process was employed for the first sample (day zero). Therefore, the changes should stem from the combined effects of freezing, storage, and thawing. Error bars are standard deviations from two chips each containing 12 microwells.