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Supporting Information

Construction of a Reusable Multi-enzyme Supramolecular Device via Disulfide Bond Locking

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Materials and Methods

The plasmids pET21a-LDH-PDZ (containing LPd gene) and pET28a-FDH-PDZlig (containing FPl gene) were constructed previously[1]. KOD-plus-mutagenesis kit was purchased from Toyobo (Osaka, Japan). Trimethylpyruvic acid (TMP), ammonium formate, and NAD(H) were purchased from Sigma-Aldrich (Shanghai, China). All other chemicals were purchased from the Sinopharm Chemical Reagent Co., Ltd. (China) and used without additional purification.

Site-directed mutagenesis

The homology model of PDZ domain associated with PDZ ligand was constructed following the protein modeling protocol of Discovery studio 2.5 (NeoTrident). The site-directed mutagenesis was performed using the KOD-plus-mutagenesis kit, following the instructions of the manufacturer. The primers used to iPCR were 5'- T TGCAAAGAGTCTCTGGTGTAAGGATCCGA-3' and 5'-CCCTTATAAACATTTC CAGCCTCTGTTTGC-3' (for MFPl), and 5'-GTGAATGCAAGATGCCGATTCTGA TTAG-3' and 5'-GGCCACCT TTGATGCTAATGCCCAG-3' (for MLPd).

Protein expression and purification

Recombinant E. coli BL21 (DE3) strains were cultured in LB medium adding 100 μg/ml ampicillin (for MLPd and LPd) or 50 μg/ml kanamycin (for MFPl and FPl) at 37°C. After OD600 reached to 0.8, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to 0.1 mM and cells were incubated at 18°C for 18 h. Cells were harvested and resuspended in 20 mM phosphate-buffered saline (PBS; pH 7.0). Then, resuspended cells were lysed by sonicating for 15 min at 0 °C. The supernatant of cell lysates was applied to an AKTA Prime system equipped with a 5-ml HisTrap FF column (GE Healthcare, Waukesha, WI, USA). Protein concentration was determined by the Bradford assay.

Activity assay

The activity of LPd, FPl, MLPd, and MFPl was measured using a SpectraMax 190 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) by monitoring the increase or decrease in absorbance at 340 nm. The standard assay mixture for formate

hydrogenase activity contained 40 mM ammonium formate, 0.1 mM NAD^+, and 100 mM phosphate buffer (pH 8.0), while for leucine dehydrogenase activity, the mixture contained 40 mM ammonium chloride, 40 mM TMP, 0.1 mM NADH, and 100 mM phosphate buffer (pH 8.0).

The enzymatic activity for the synthesis of L-tert-leucine was determined by measuring the conversion ratio of TMP. The reaction mixture contained 40 mM ammonium formate, 40 mM TMP, 0.1 mM NAD^+, and 100 mM phosphate buffer (pH 8.0). After reaction at 30°C and 200 rpm for 1 h, a 20-μl sample was removed and analyzed by reverse-phase high-performance liquid chromatography using an 1100 Series chromatograph and SB-Aq column (250 × 4.6 mm, 5 μm; Agilent Technologies, Inc., Santa Clara, CA, USA) at 210 nm.

In vitro supramolecular self-assembly and air oxidation

Freshly purified MLPd (2ml, 20μM) and MFPl (2ml, 20μM) were mixed in a 50-ml shake flask and incubated at 100 rpm, 4 °C for 5-8 h to induce the formation of multienzyme supramolecular devices completely. Then the disulfide-locked LPd-FPl MESDs were collected by centrifuging at 10000×g for 10 min, and the precipitated assemblies were subjected to SDS-PAGE under conditions of reduction and non-reduction.

SDS-PAGE

The gel of SDS-PAGE included separating gel and stacking gel. The separating gel (12% acrylamide/bisacrylamide) contained 1.75 mL of H_2O, 2 mL of 30% acrylamide/bisacrylamide, 1.25 mL of 4×separating gel buffer（1.5 M Tris-HCl, 0.4% SDS, pH 8.8), 30 μL of 10% ammonium persulfate, and 3 μL of TEMED. The stacking gel (5% acrylamide/bisacrylamide) contained 1.25 μL of H_2O, 335 μL of 30% acrylamide/bisacrylamide, 500 μL of 4×stacking gel buffer（0.5 M Tris-HCl, 0.4% SDS, pH 6.8), 15 μL of 10% APS, and 3 μL of TEMED. The samples were incubated with reduced loading buffer (containing 2% beta-mercaptoethanol, 5% glycerin, 1‰ bromophenol blue, 15mM SDS, and 12 mM Tris-HCl, pH6.8) and non-reduced loading buffer (reduced loading buffer without beta-mercaptoethanol).

Field-emission scanning electron microscopy

For field-emission scanning electron microscopy, samples were prepared by dropping 10 μl solution onto freshly cleaved mica for 10 min, washing with deionized water, and drying under air. Images were collected on an SU4800 scanning electron microscope (Hitachi, Tokyo, Japan) operated at 1 kV under deceleration mode.

The reusability of disulfide-locked LPd-FPl MESDs

The reusability of the disulfide-locked LPd-FPl MESDs under conditions of repeated centrifuging isolation (10000×g, 3 min) and reuse was investigated under conditions similar to those employed for the activity assay of synthesis of L-tert-leucine. The residual activity of the disulfide-locked LPd-FPl MESDs after each cycle was normalized to the initial value (the initial activity was defined as 100%).

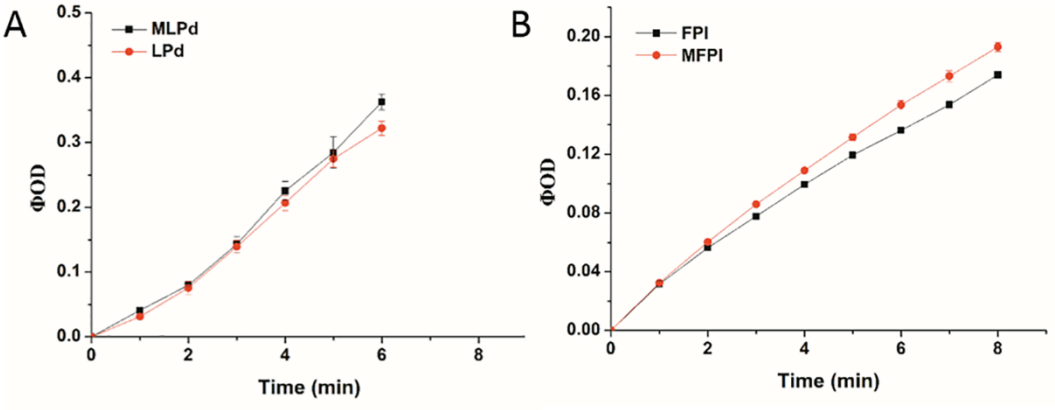
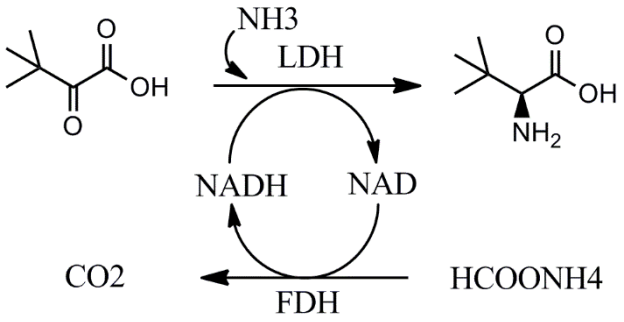


Figure S1 Enzyme activity assay for (A) MLPd and LPd, and (B) MFPl and FPl. ΦOD, absolute value of optical density changes at 340 nm. Each data point represents the mean ± standard deviation of three measurements.



Fgiure S2 Reaction scheme for conversion of trimethylpyruvic acid to L-tert-leucine. FDH represents formate dehydrogenase, and LDH represents leucine dehydrogenase.

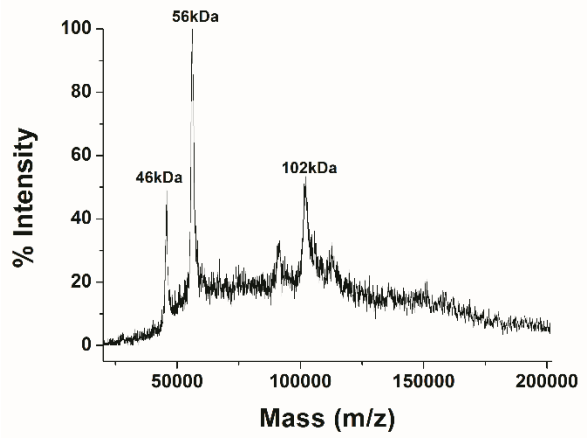


Figure S3. Results of Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry. The samples was prepared by treating disulfide-locked LPd-FPl MESDs with denaturing buffer (5% glycerin, 15mM SDS) and desalinated by ultrafiltration. The mass spectrometer 4800 Plus MALDI TOF/TOF MASS Analyzer (AB SCIEX, USA) equipped with a Nd:YAG laser emitting at 355nm was set to perform data acquisition in linear positive ion mode. 4000 series Explorer (AB SCIEX, USA) were used for data acquisition and processing. The acceleration voltage was set to 20 kV and the extraction delay time used was 1500 ns.

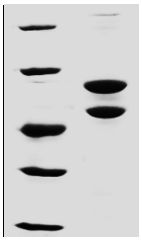


Figure S4. Protein marker (left, 116, 66, 45, 35, 25 kDa) and the results of non-reduced protein sample applied to SDS-PAGE (right). Protein sample was the precipitated assemblies formed by mixing MLPd and MFPl under condition of air-free.

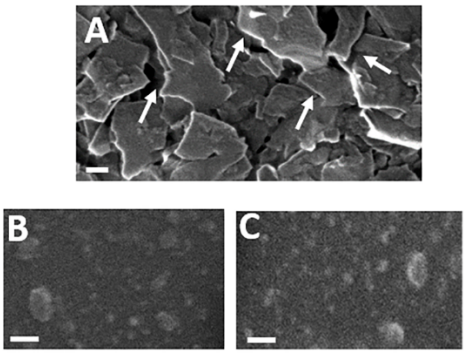


Figure S5. SEM images for samples of unlocked LPd-FPl MESDs (A), MLPd (B), and MFPl (C). Scale bar was 100nm. The boundaries of each layer-like structure were clear, which suggested that there was no linkage between layer-like structures (white arrows in A).

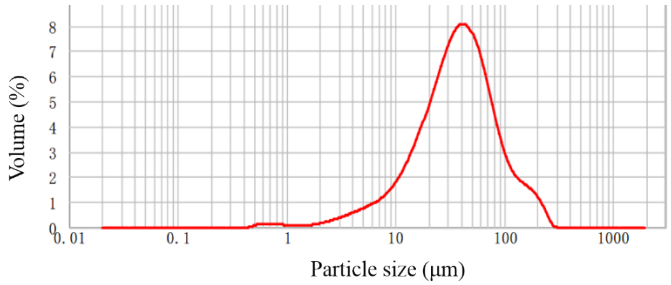


Figure S6. The results of particle size distribution of disulfide-locked LPd-FPl MESDs. Size distribution analysis was carried out by using a MS 2000 laser particle size analyzer (Malvern, UK). The samples were prepared by suspending locked LPd-FPl MESDs in PBS (pH7.0).

References

1 X. Gao, S. Yang, C. Zhao, Y. Ren and D. Wei, *Angewandte Chemie*, 2014, **126**, 14251.