

Supporting information

Specific Immobilization of D-Amino Acid Oxidase on Hematin-functionalized Support Mimicking Multi-enzyme Catalysis

Jian Sun^a, Kun Du^a, Xiaoqiang Song^a, Qian Gao^a, Hao Wu^b, Jingjing Ma^a, Peijun Ji^b,
Wei Feng^{a*}

^aDepartment of Biochemical Engineering, ^bDepartment of Chemical Engineering,
Beijing University of Chemical Technology, Beijing, 100029, China

Gene constructions and cloning for an elastin-like polypeptide (ELP). A 20-repeat polypeptide of Val-Pro-Gly-Xaa-Gly was synthesized in PUC57 plasmid by the genewiz company (Suzhou, China). (VPGXG)₂₀ was used as the monomer for synthesis of (VPGXG)₆₀ and Xaa was chosen to be Val:Ala:Gly in a 5:3:2 ratio.¹ The gene sequence of the monomer (VPGXG)₂₀ is listed in [Table S1](#) (see supporting information).

PUC57-(VPGXG)₂₀ was linearized with *Pfl*MI (2 μL *Pfl*MI I; 3 μL 10×K buffer; 25 μL PUC57-(VPGXG)₂₀ at at 37 °C for 3 h), enzymatically dephosphorylated with alkaline phosphatase, and then purified using a DNA extraction kit (Omega Bio-tek). Another aliquot of the plasmid was codigested with *Pfl*MI and *Bgl* I restriction endonucleases to generate the free (VPGXG)₂₀ insert (2 μL *Pfl*MI I; 2 μL *Bgl* I; 5 μL 10×K buffer; 41 μL PUC57-(VPGXG)₂₀). After digestion, the reaction products were separated by agarose gel electrophoresis, and the insert was purified using a DNA extraction kit (Omega Bio-tek).

The monomers were then ligated to the linearized vector (0.4 μL T4 DNA ligase, 2

* Department of Biochemical Engineering, Beijing University of Chemical Technology, Beijing, 100029, China.

E-mail: fengwei@mail.buct.edu.cn.

μL 10 \times ligation buffer; 2 μL PUC57-(VPGXG)₂₀, 15.6 μL insert, incubated at 22 °C for 20 min). A 10 μL portion of the ligation mixture was combined with 100 μL of chemically competent *Escherichia coli* cells (DH5 α), and the cells were transformed by heat shock (30 min on ice, 90 s 42 °C, 3 min on ice). After addition of 900 μL LB medium, the cells were cultured for 45 min, spread on LB medium agar plates supplemented with ampicillin (50 $\mu\text{g}/\text{mL}$), and incubated at 37 °C. The transformants were verified by their digestions with diagnostic restriction endonucleases and confirmed by DNA sequencing (BGI Tech). The result of this process was a (VPGXG)₄₀ insert in the pUC-57 vector. Subsequent additional round of recursive directional ligation proceed identically for (VPGXG)₆₀. The plasmid pET28a was codigested BamH I and Hind III restriction endonucleases. The pUC-57 vector harboring the (VPGXG)₆₀ gene was codigested BamH I and Hind III restriction endonucleases, and the resulting fragment was ligated into the plasmid pET28a to construct the expression vector pET28a/(VPGXG)₆₀ in *Escherichia coli*. [Fig. S1a](#) shows the SDS-PAGE analysis for the ELP (VPGXG)₆₀ (see supporting information). The ELP was approximately 29.5 kDa in size, in agreement with the theoretically predicted molecular weight the molecular weight. The purified ELP can be reversed from a cloudy suspension to a clear solution at a low temperature (4 °C) ([Fig. S1b](#)).

Expression vector construction. The DAAO gene was amplified by PCR using Pfu DNA polymerase with genomic DNA of *Trigonopsis variabilis* as template using the paired primers ([Table S2](#)). The DAAO gene was purified using a DNA extraction kit (Omega Bio-tek) and was then codigested codigested Nco I and BamH I restriction endonucleases. The resulting fragment was ligated into the plasmid pET28a/(VPGXG)₆₀, which has been codigested with Nco I and BamH I restriction endonucleases. Thus the expression vector pET28a/(VPGXG)₆₀-DAAO in *Escherichia coli*. was constructed.

Table S1. ELP monomer

The gene sequence of the monomer (VPGXG)₂₀ with a restriction site of SacI at 5' terminal and a restriction site of Sall at 3' terminal

5'GGATCCGAGCTCCATATGGGCCACGGCGTGGGTGTTCCGGGCGTGGGTG
TTCCGGGTGGCGGTGTGCCGGGCGCAGGTGTTCCCTGGTGTAGGTGTGCCG
GGTGTGGTGTGCCGGGTGTTGGTGTACCAGGTGGCGGTGTTCCGGGTGC
AGGCGTTCGGGTGGCGGTGTGCCGGGCGTGGGTGTTCCGGGCGTGGGTG
TTCCGGGTGGCGGTGTGCCGGGCGCAGGTGTTCCCTGGTGTAGGTGTGCCG
GGTGTGGTGTGCCGGGTGTTGGTGTACCAGGTGGCGGTGTTCCGGGTGC
AGGCGTTCGGGTGGCGGTGTGCCGGGCGGGCTGGTCGACAAGCTT3'

Table S2. DAAO gene sequence and primers

ATGGCTAAAATCGTTGTTATTGGTGCCGGTGTTGCCGGTTTAACTACAGCT
CTTCAACTTCTTCGTAAAGGACATGAGGTTACAATTGTGTCCGAGTTTACG
CCCGGTGATCTTAGTATCGGATATACCTCGCCTTGGGCAGGTGCCAACTGG
CTCACATTTTACGATGGAGGCAAGTTAGCCGACTACGATGCCGTCTCTTAT
CCTATCTTGCGAGAGCTGGCTCGAAGCAGCCCCGAGGCTGGAATTGACT
CATCAACCAACGCTCCCATGTTCTCAAGCGTGATCTTCCTAAACTGGAAGG
TGCCATGTCGGCCATCTGTCAACGCAACCCCTGGTTCAAAAACACAGTCG
ATTCTTTTCGAGATTATCGAGGACAGGTCCAGGATTGTCCACGATGATGTG
GCTTATCTAGTCGAATTTGCTTCCGTTTGTATCCACACCGGAGTCTACTTG
AACTGGCTGATGTCCCAATGCTTATCGCTCGGCGCCACGGTGGTTAAACGT
CGAGTGAACCATATCAAGGATGCCAATTTACTACACTCCTCAGGATCACG
CCCCGACGTGATTGTCAACTGTAGTGGTCTCTTTGCCCGGTTCTTGGGAGG
CGTCGAGGACAAGAAGATGTACCTATTCGAGGACAAGTCGTCCTTGTTT
GAAACTCTCTTCCTTTTATGGCCTCCTTTTCCAGCACTCCTGAAAAAGAAA
ATGAAGACGAAGCTCTATATATCATGACCCGATTCGATGGTACTTCTATCA
TTGGCGGTTGTTTCCAACCAACAACCTGGTCATCCGAACCCGATCCTTCTC
TCACCCATCGAATCCTGTCTAGAGCCCTCGACCGATTCCCGGAACCTGACCA
AAGATGGCCCTCTTGACATTGTGCGCGAATGCGTTGGCCACCGTCCTGGTA
GAGAGGGCGGTCCCCGAGTAGAATTAGAGAAGATCCCCGGCGTTGGCTTT
GTTGTCCATAACTATGGTGCCGCGGTGCTGGTTACCAGTCCTCTTACGGC
ATGGCTGATGAAGCTGTTTCTTACGTCGAAAGAGCTCTTACTCGTCCAAAC
CTT

The primers used for the amplification of the DAAO gene, which were designed based on the gene sequence of the DAAO (GenBank accession AY514426). The underlines indicates Nco I and BamH I restriction sites, respectively.

5'GCGCCATGGCTAAAATCGTTGTTATTG3'

5'ATGGATCCAAGGTTTGGACGAGTAAGAG3'

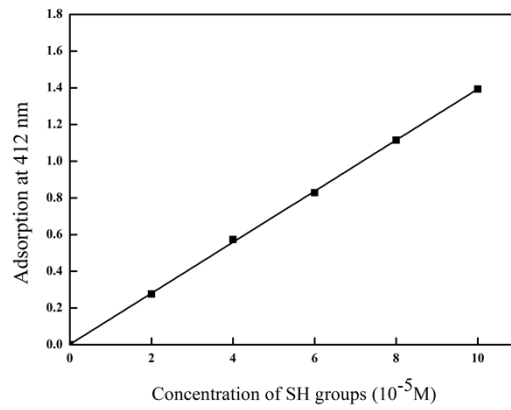


Fig. S1. Standard curve for SH group

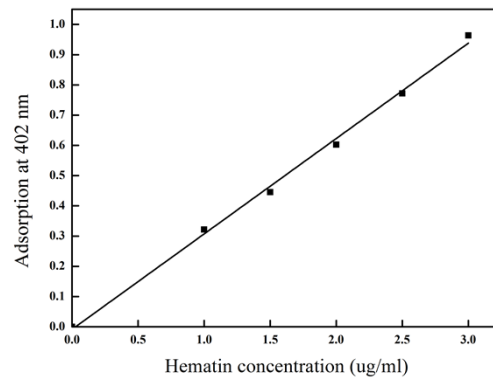


Fig. S2. Standard curve for hematin

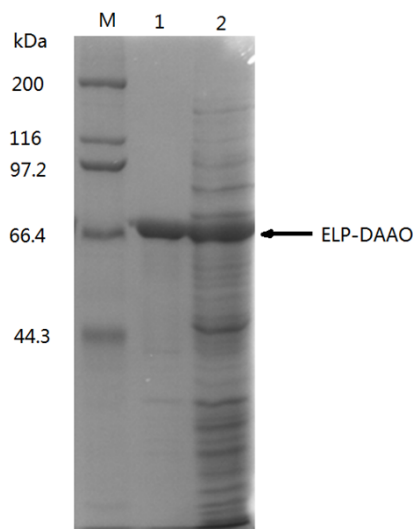


Fig. S3. Analysis of purified ELP-DAAO by SDS-PAGE.

Lane M: molecular mass marker (KDa); lane 1 is for the supernatants from the third round of inverse transition cycling; lane 2 is for total proteins.

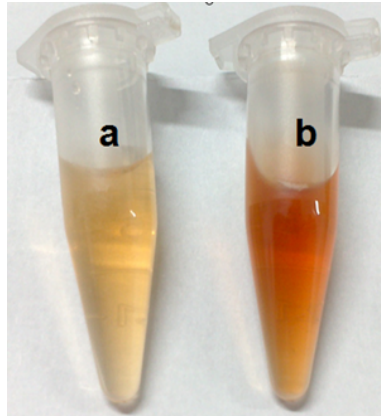


Fig. S4. Deamination of D-alanine catalyzed by free ELP-DAAO (a) and immobilized ELP-DAAO (b).

Reaction conditions: equivalent amount of enzyme was used, 0.1 mg/ml ELP-DAAO; D-alanine (1.30 mM) was dissolved in 1 ml PBS buffer (pH8.0, 50 mM). The reactions were carried out at 30 °C for 3 min. 200 μ l trichloroacetic acid solution (20 % w/v) was then added to the tubes to terminate the reactions. The solutions were centrifuged. 100 μ l 2,4-dinitrophenylhydrazine (DNPH) solution (10 mM) was added to the supernatants, incubated at 37 °C for 10 min, and then 700 μ l NaOH (1.5 M) was added.

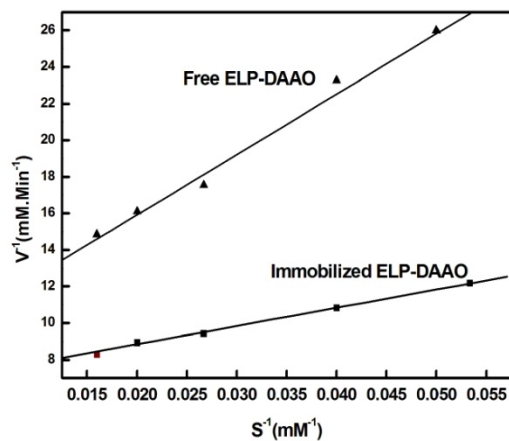


Fig. S5. 1/Vmax versus 1/C for the free and immobilized enzymes

The concentration of substrate ranged from 20 to 63 mM.

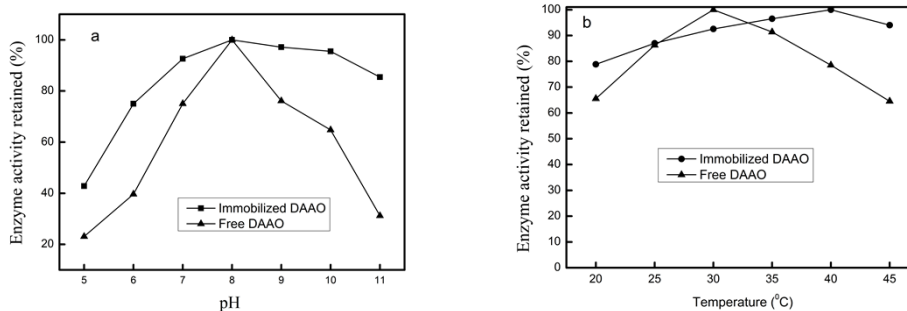


Fig. S6. Effect of pH (a) and temperature (b) on the retaining of enzymatic

activity for immobilized and free enzymes

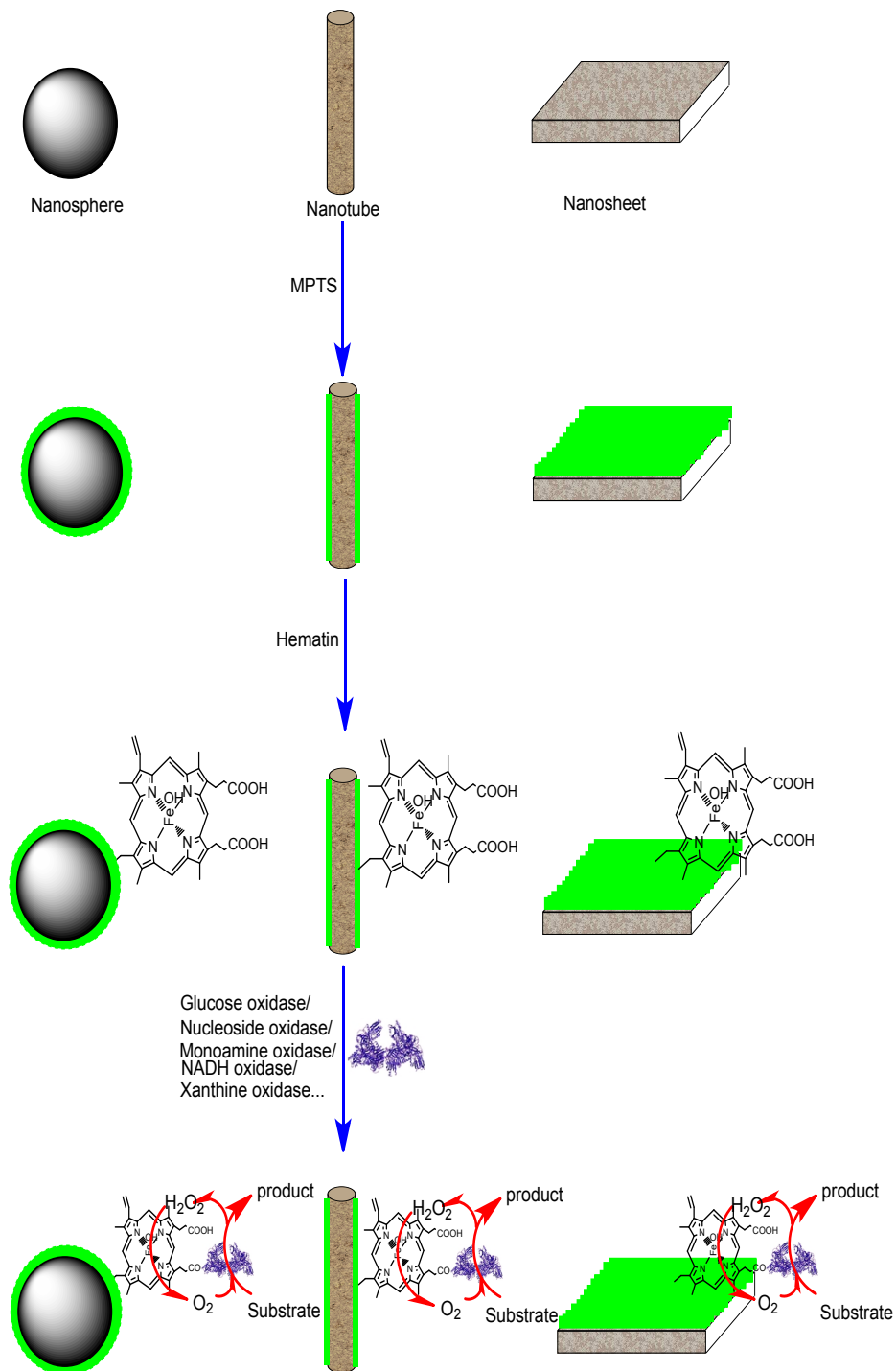


Fig. S7. Schematic showing the extension of the enzyme immobilization methodology to oxidases and supports

Reference

1. D. E. Meyer, A. Chilkoti, *Biomacromolecules*, 2002, **3**, 357-367