Supplementary Materials: Immobilization of Genetically-Modified D-Amino Acid Oxidase and Catalase on Carbon Nanotubes to Improve the Catalytic Efficiency

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1. 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 the 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.

PUC57-$(VPGXG)_{20}$ was linearized with *Pfl*MI (2 μL *PflM I*; 3 μL 10 × K buffer; 25 μL PUC57-$(VPGXG)_{20}$ at 37 °C for 3 h), enzymatically dephosphorylated with alkaline phosphatase, and then purified using a DNA extraction kit (Omega Bio-tek, Inc., Norcross, GA, USA). Another aliquot of the plasmid was co-digested with *Pfl*MI and *Bgl* I restriction endonucleases to generate the free $(VPGXG)_{20}$ insert (2 μL PflM 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 μL 10× 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 at 42 °C, 3 min on ice). After the addition of 900 μL of LB medium, the cells were cultured for 45 min, spread on LB medium agar plates supplemented with ampicillin (50 μg/mL), and incubated at 37 °C. The transformants were verified by their digestion 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. A subsequent additional round of recursive directional ligation proceeded identically for $(VPGXG)_{60}$. The plasmid pET28a was co-digested with BamH I and Hind III restriction endonucleases. The pUC-57 vector harboring the $(VPGXG)_{60}$ gene was co-digested with 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*.

2. Expression Vector Construction

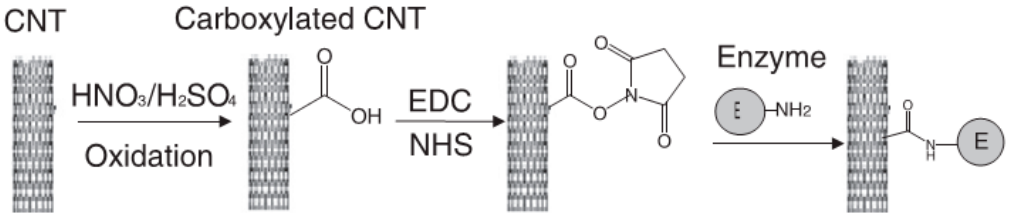
The CAT gene was amplified by PCR using Pfu DNA polymerase, with primers GCCCATGGGCTCAAATAAACTGACAAC and GCCGAGCTCAGAATCTTTTTTAATCGG. The CAT gene was purified using a DNA extraction kit (Omega Bio-tek) and was then co-digested with Nco I and SacI restriction endonucleases. The resulting fragment was ligated into the plasmid pET28a/$(VPGXG)_{60}$, which was co-digested with Nco I and SacI restriction endonucleases.

3. Protein Purification and Turbidity Measurements

Proteins were purified using inverse transition cycling [1]. *Escherichia coli* cells were harvested by centrifugation at 4°C and re-suspended in 50 mL of PBS buffer. Cells were lysed by ultrasonic disruption on ice, and the lysate was centrifuged at 10,000 *g* at 4 °C for 30 min to remove cell debris. The supernatant was transferred to a fresh tube, and sodium chloride solution (3 M) was added and mixed with the sample. The resulting sample was heated to 30 °C for 10 minutes followed by centrifugation at 30 °C for 10 minutes. The purification process was repeated three times.

Table S1. Elastin-like polypeptide (ELP) monomer.

The gene sequence of the monomer $(VPGXG)_{20}$ with a restriction site of SacI at 5' terminal and a restriction site of SalI at 3' terminal is
5'GGATCCGAGCTCCATATGGGCCACGGCGTGGGTGTTCCGGGCGTGGGTGTTCCGG GTGGCGGTGTGCCGGGCGCAGGTGTTCCTGGTGTAGGTGTGCCGGGTGTTGGTGTG CCGGGTGTTGGTGTACCAGGTGGCGGTGTTCCGGGTGCAGGCGTTCCGGGTGGCGG TGTGCCGGGCGTGGGTGTTCCGGGCGTGGGTGTTCCGGGTGGCGGTGTGCCGGGC GCAGGTGTTCCTGGTGTAGGTGTGCCGGGTGTTGGTGTGCCGGGTGTTGGTGTACC AGGTGGCGGTGTTCCGGGTGCAGGCGTTCCGGGTGGCGGTGTGCCGGGCGGGCTG GTCGACAAGCTT3'



Scheme S1. Schematic presentation for covalent immobilization of enzymes on carbon nanotubes (CNTs). EDC: *N*-ethyl-*N*-(3-(dimethylamino)propyl) carbodiimide hydrochloride; NHS: *N*-hydroxysuccinimide.

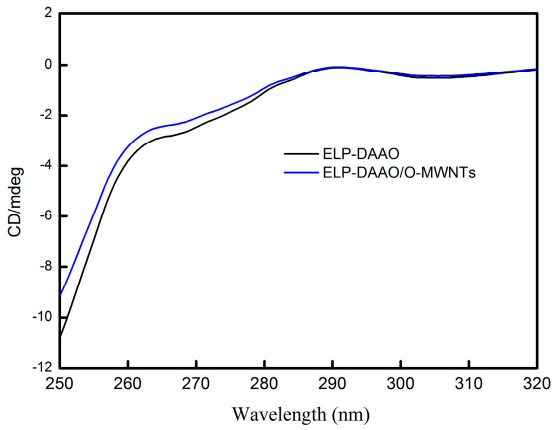


Figure S1. Near-UV circular dichroism (CD) spectra of ELP-DAAO (black) and immobilized ELP-DAAO (blue). The near-UV CD spectra were measured at 250–320 nm, with protein concentrations of 1 mg/mL. DAAO: D-Amino Acid Oxidase; O-MWNTs: Oxidized Multi-Walled Carbon Nanotubes.

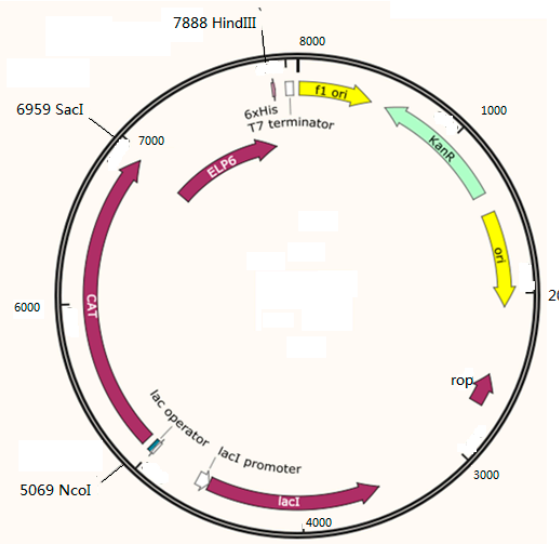


Figure S2. Schematic presentation of constructed vector for ELP-CAT. CAT: Catalase.

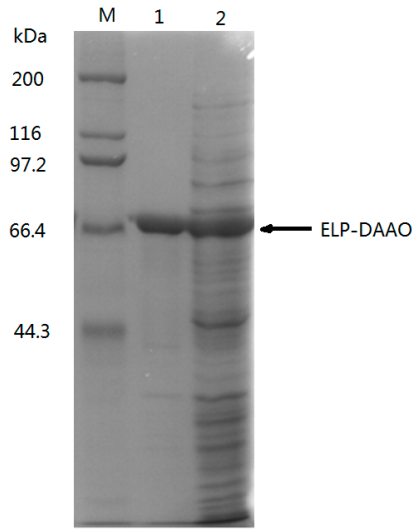


Figure S3. Analysis of purified ELP-DAAO fusion protein by SDS-PAGE. Lane M: molecular mass marker (KDa); Lane 1 is for the supernatants from the third round of inverse transition cycling; Lane 2 is for total proteins.

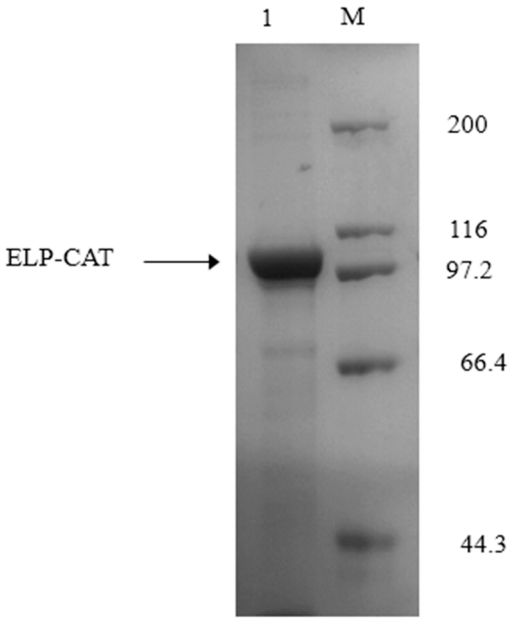


Figure S4. Analysis of purified ELP-CAT fusion protein by SDS-PAGE. Lane M: molecular mass marker (KDa); Lane 1 is for the supernatants from the third round of inverse transition cycling

Reference

1. Meyer, D. E.; Chilkoti, A. Genetically encoded synthesis of protein-based polymers with precisely specified molecular weight and sequence by recursive directional ligation: examples from the elastin-like polypeptide system. *Biomacromolecules* **2002**, *3*, 357–367.