Supplementary Materials: Co-Immobilization of Superoxide Dismutase with Catalase on Soft Microparticles Formed by Self-Assembly of Amphiphilic Poly(Aspartic Acid)

2. Results and Discussion

2.1. Purification of the Fusion Enzyme

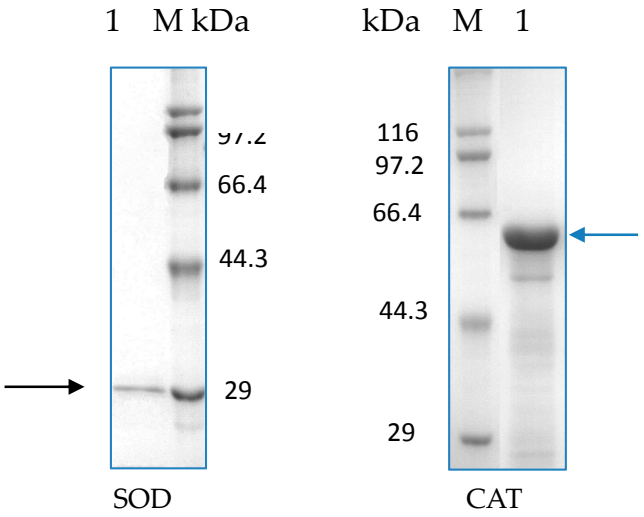

Figure S1. Analysis of purified SOD (a) and CAT (b) by SDS-PAGE. Lane M: molecular mass marker (KDa); Lane 1 is for the purified proteins. SOD: superoxide dismutase; CAT: catalase

3. Experimental Section

3.1. Materials

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.

PUC57-$(VPGXG)_{20}$ was linearized with *Pfl*MI (2 μL *PflM I*; 3 μL 10×K buffer; 25 μL PUC57-$(VPGXG)_{20}$ at 37 ^{0}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 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 (DH5α), and the cells were transformed by heat shock (30 min on ice, 90 s at 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 μg/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 finally constructed vector is designated as pUC-57-ELP.

The plasmid pET28a was codigested with BamH I and Hind III restriction endonucleases. The pUC-57-ELP vector harboring the ELP gene was codigested with BamH I and Hind III restriction endonucleases, and the resulting fragment was ligated into the plasmid pET28a to construct the expression vector pET28a-ELP in *Escherichia coli*.

3.3. Co-Immobilization of SOD-ELP and CAT-ELP on HPASP

Expression Vector Construction

The SOD gene was amplified by PCR using LA Taq DNA polymerase with primers CATGCCATGGCTTACGAACTTCCAG and GGAATTCCATATGTTTTGCTTCG CTGTATAG. The SOD gene was purified using a DNA extraction kit (Omega Bio-tek) and was then codigested with NdeI and NcoI restriction endonucleases. The resulting fragment was ligated into the plasmid pET28a-ELP, which has been codigested with NdeI and NcoI restriction endonucleases. Thus the expression vector pET28a-SOD-ELP in *Escherichia coli* was constructed.

The CAT gene was amplified by PCR using Pfu DNA polymerase, with primers GCCCATGGGCTCAAATAAACTGACAAC and GCCGAGCTCAGAATCTTTTTT AATCGG. The CAT gene was purified using a DNA extraction kit (Omega Bio-tek) and was then codigested with Nco I and SacI restriction endonucleases. The resulting fragment was ligated into the plasmid pET28a-ELP, which has been codigested with Nco I and SacI restriction endonucleases. Thus the expression vector pET28a-CAT-ELP in *Escherichia coli* was constructed.

Synthesis of Hexadecylamine Modified Poly(aspartic acid)

The hexadecylamine-modified poly(aspartic acid) (HPASP) was synthesized according to following procedure. L-aspartic acid (5 g) was suspended in a flask in the presence of phosphoric acid (3 mL) and stirred at 190 ^{0}C. After 2 h, the reaction mixture was precipitated in excess methanol and successively washed with water until the pH of the suspension became neutral. The precipitate was dissolved in DMF, and the mixture was incubated at 40 ^{0}C for 2 h. The mixture was then filtered, and the filtrate was precipitated in excess methanol. The precipitate was dried at 80 ^{0}C in vacuo. The synthesized PSI (950 mg) was dissolved in DMF (12 mL). Hexadecylamine (700 mg) dissolved in DMF was added to the mixture rapidly. The reaction mixture was stirred at 70 ^{0}C for 24 h. The reaction mixture was cooled to room-temperature. Insoluble product was filtered out. The clear solution was added dropwise to 1N NaOH solution to hydrolyze remaining succinimide unit of PSI. After stirring for 3 h in an ice water bath, the reaction mixture was precipitated in excess methanol twice. The precipitate was filtered and washed with methanol and then dried in vacuo at 60 ^{0}C.

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 SalI at 3' terminal is
5'GGATCCGAGCTCCATATGGGCCACGGCGTGGGTGTTCCGGGCGTGGGTGTTCCGGGTGGCGGTGTGC
CGGGCGCAGGTGTTCCTGGTGTAGGTGTGCCGGGTGTTGGTGTGCCGGGTGTTGGTGTACCAGGTGGC
GGTGTTCCGGGTGCAGGCGTTCCGGGTGGCGGTGTGCCGGGCGTGGGTGTTCCGGGCGTGGGTGTTCC
GGGTGGCGGTGTGCCGGGCGCAGGTGTTCCTGGTGTAGGTGTGCCGGGTGTTGGTGTGCCGGGTGTTG
GTGTACCAGGTGGCGGTGTTCCGGGTGCAGGCGTTCCGGGTGGCGGTGTGCCGGGCGGGCTGGTCGAC
AAGCTT3'

Reference

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.