Supplementary Materials: Enhancing the Enzymatic Activity of a Heme-Dependent Peroxidase through Genetic Modification

Wei Liu, Rong Li, Dan Liu and Wei Feng

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 [18]. The gene sequence of the monomer (VPGXG)20 is listed in Table S1.

PUC57-(VPGXG)20 was linearized with PflMI (2 μL PflMI; 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, Shanghai, China). Another aliquot of the plasmid was codigested with PflMI and BglI restriction endonucleases to generate the free (VPGXG)20 insert (2 μL PflMI; 2 μL BglI; 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, Beijing, China), 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 Lysogeny broth (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, Shenzhen, China). 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 pET-28a was codigested with BamH I and Hind III restriction endonucleases. The pUC-57 vector harboring the (VPGXG)60 gene was codigested with BamH I and Hind III restriction endonucleases, and the resulting fragment was ligated into the plasmid pET-28a to construct the expression vector pET-28a-ELP in *Escherichia coli*.

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
5'GGATCCGAGCTCCATATGGGCCACGGCGTGGGTGTTCCGGGCGTGGGTGTTCCGGGTGG
CGGTGTGCCGGGCGCAGGTGTTCCTGGTGTAGGTGTGCCGGGTGTTGGTGTGCCGGGTGT
TGGTGTACCAGGTGGCGGTGTTCCGGGTGCAGGCGTTCCGGGTGGCGGTGTGCCGGGCGT
GGGTGTTCCGGGCGTGGGTGTTCCGGGTGGCGGTGTGCCGGGCGCAGGTGTTCCTGGTGT
AGGTGTGCCGGGTGTTGGTGTGCCGGGTGTTGGTGTACCAGGTGGCGGTGTTCCGGGTGC
AGGCGTTCCGGGTGGCGGTGTGCCGGGCGGGCTGGTCGACAAGCTT3'

Table S2. The gene of the heme-dependent peroxidase.

1	AAG CTT GGA TCC GTG AGC AAC GGC CGT GGT
1	Lys Leu Gly Ser Val Ser Asn Gly Arg Gly
31	CAT GCC GCC GCA CCG GGC GGG GGG CAC TCG
11	His Ala Ala Ala Pro Gly Gly Gly His Ser
61	CCG CTG CTG CAA CCG CAA CTG CTG TTC ATG
21	Pro Leu Leu Gln Pro Gln Leu Leu Phe MET
91	CCT CCG GTG GGC CAC GCG TAC GAG ACC CCG
31	Pro Pro Val Gly His Ala Tyr Glu Thr Pro
121	TCC GAG GAG GTG CCG CAC ACC ACC GGG GCC
41	Ser Glu Glu Val Pro His Thr Thr Gly Ala
151	GCC GAC CGG GAC GCG CCG GAC TAC GAC CTC
51	Ala Asp Arg Asp Ala Pro Asp Tyr Asp Leu
181	TTC GGC GAA CGC CCG GTC GAG GCG CAG CGG
61	Phe Gly Glu Arg Pro Val Glu Ala Gln Arg
211	CTG TTC TGG TAC CGC TGG ATC GCC GGC CAC
71	Leu Phe Trp Tyr Arg Trp Ile Ala Gly His
241	CAG ATC TCG TTC GTG CTC TGG CGG GCC ATG
81	Gln Ile Ser Phe Val Leu Trp Arg Ala MET
271	GGG GAC ATC CTG TGG CAC CAC CCG CAT GAC
91	Gly Asp Ile Leu Trp His His Pro His Asp
301	GTG CCT GGC GCC CGC GAA CTC GAC GTG CTG
101	Val Pro Gly Ala Arg Glu Leu Asp Val Leu
331	ACC GCC TGC GTC GAC GGT TAC AGC GCG ATG
111	Thr Ala Cys Val Asp Gly Tyr Ser Ala MET
361	CTG CTC TAC TCG GCC ACC GTC CCG CGT GCC
121	Leu Leu Tyr Ser Ala Thr Val Pro Arg Ala
391	CAC TAC CAC TCC TAC ACC CGT GCG CGC ATG
131	His Tyr His Ser Tyr Thr Arg Ala Arg MET
421	GCG CTG CAG CAC CCG TCG TTC AGC GGC GCG
141	Ala Leu Gln His Pro Ser Phe Ser Gly Ala
451	TGG GCG CCG GAC TAC CGG CCG ATC CGC CGG
151	Trp Ala Pro Asp Tyr Arg Pro Ile Arg Arg
481	CTC TTC CGC AAC CGC TTG CCG TGG CAG GGC
161	Leu Phe Arg Asn Arg Leu Pro Trp Gln Gly
511	GAT CCG TCG TGC CGT GCC CTG GGC GAG GCG
171	Asp Pro Ser Cys Arg Ala Leu Gly Glu Ala
541	GTC GCG CGC AAC GGC GTG ACC CAC GAC CAC
181	Val Ala Arg Asn Gly Val Thr His Asp His
571	ATC GCC AAC CAC CTC GTG CCT GAC GGG CGG
191	Ile Ala Asn His Leu Val Pro Asp Gly Arg
601	TCC CTG CTG CAG CAG TCC GCC GGC GCA CCG
201	Ser Leu Leu Gln Gln Ser Ala Gly Ala Pro

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631         GGC GTG ACC GTG TCC CGG GAG AAG GAG GAC
211         Gly Val Thr Val Ser Arg Glu Lys Glu Asp
661         CTC TAC GAC AAC TTC TTC CTG ACC GTC CGG
221         Leu Tyr Asp Asn Phe Phe Leu Thr Val Arg
691         CGG CCG GTC AGC CAC GCC GAA CTC GTC GCG
231         Arg Pro Val Ser His Ala Glu Leu Val Ala
721         CAG CTG GAC GCG CGC GTC ACG GAG GTC GCG
241         Gln Leu Asp Ala Arg Val Thr Glu Val Ala
751         GCG GAC CTC CGG CAC AAC GGG CTC TAC CCG
251         Ala Asp Leu Arg His Asn Gly Leu Tyr Pro
781         AAC GTC GAC GGT CGC CAC CAC CCG GTC GTC
261         Asn Val Asp Gly Arg His His Pro Val Val
811         ACC TGG CAG TCG GAC GGT GTG ATG GGG TCG
271         Thr Trp Gln Ser Asp Gly Val MET Gly Ser
841         CTG CCG ACC GGT GTC CTG CGG ACG CTG AAC
281         Leu Pro Thr Gly Val Leu Arg Thr Leu Asn
871         CGG GCG ACG CGG ATG GTC GCG CAG ACG CGC
291         Arg Ala Thr Arg MET Val Ala Gln Thr Arg
901         CTC GAG GAA GCC CGG TCA GAG CTC GCG GCC
301         Leu Glu Glu Ala Arg Ser Glu Leu Ala Ala
 931         GC
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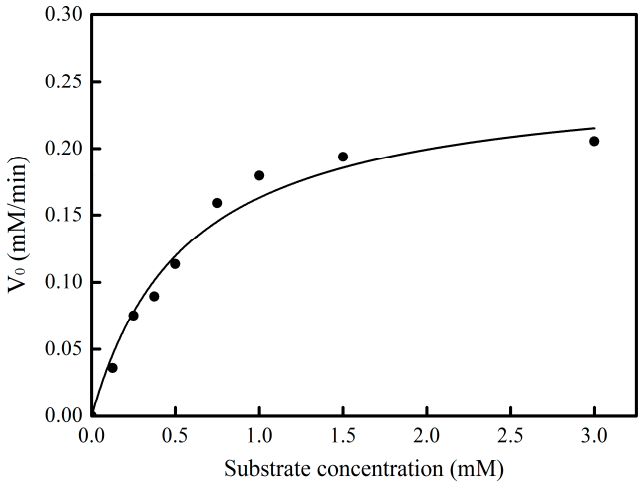

Figure S1. Plot of the initial rate of reaction (V_0) as a function of the substrate concentration for HDP-ELP.

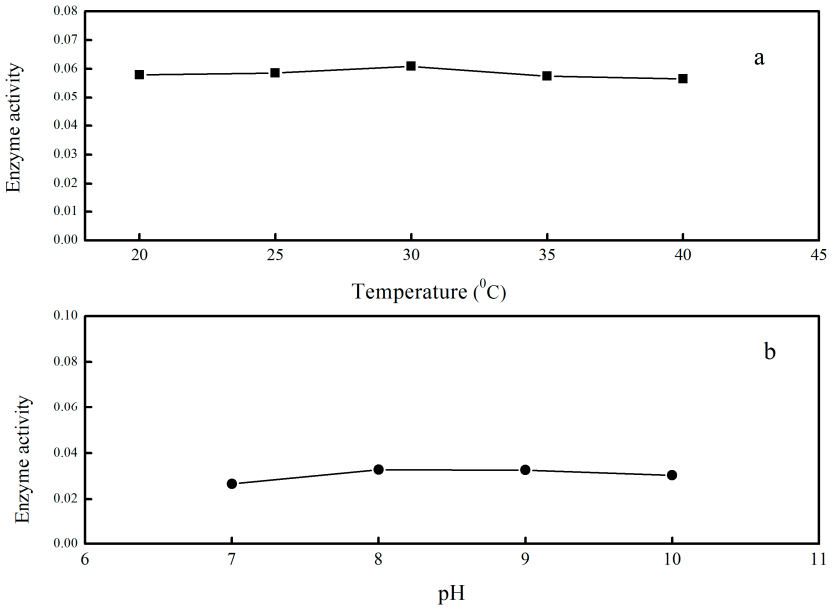

Figure S2. Effect of temperature and pH conditions on the enzymatic activity for HDP-ELP.