# 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  $\mu$ L PflMI; 3  $\mu$ L 10× K buffer; 25  $\mu$ L 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  $\mu$ L PflMI; 2  $\mu$ L BglI; 5  $\mu$ L 10× K buffer; 41  $\mu$ L 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 ( $0.4 \ \mu L T4 DNA ligase, 2 \ \mu L 10 \times ligation$  buffer; 2  $\mu L PUC57$ -(VPGXG)20, 15.6  $\mu L$  insert, incubated at 22 °C for 20 min). A 10  $\mu L$  portion of the ligation mixture was combined with 100  $\mu L$  of chemically competent *Escherichia coli* cells (DH5 $\alpha$ , 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  $\mu L$  Lysogeny broth (LB) medium, the cells were cultured for 45 min, spread on LB medium agar plates supplemented with ampicillin (50  $\mu$ g/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, 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

## 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

GGC	GTG	ACC	GTG	TCC	CGG	GAG	AAG	GAG	GAC
Gly	Val	Thr	Val	Ser	Arg	Glu	Lys	Glu	Asp
CTC	TAC	GAC	AAC	TTC	TTC	CTG	ACC	GTC	CGG
Leu	Tyr	Asp	Asn	Phe	Phe	Leu	Thr	Val	Arg
CGG	CCG	GTC	AGC	CAC	GCC	GAA	CTC	GTC	GCG
Arg	Pro	Val	Ser	His	Ala	Glu	Leu	Val	Ala
CAG	CTG	GAC	GCG	CGC	GTC	ACG	GAG	GTC	GCG
Gln	Leu	Asp	Ala	Arg	Val	Thr	Glu	Val	Ala
GCG	GAC	CTC	CGG	CAC	AAC	GGG	CTC	TAC	CCG
Ala	Asp	Leu	Arg	His	Asn	Gly	Leu	Tyr	Pro
AAC	GTC	GAC	GGT	CGC	CAC	CAC	CCG	GTC	GTC
Asn	Val	Asp	Gly	Arg	His	His	Pro	Val	Val
ACC	TGG	CAG	TCG	GAC	GGT	GTG	ATG	GGG	TCG
Thr	Trp	Gln	Ser	Asp	Gly	Val	MET	Gly	Ser
CTG	CCG	ACC	GGT	GTC	CTG	CGG	ACG	CTG	AAC
Leu	Pro	Thr	Gly	Val	Leu	Arg	Thr	Leu	Asn
CGG	GCG	ACG	CGG	ATG	GTC	GCG	CAG	ACG	CGC
Arg	Ala	Thr	Arg	MET	Val	Ala	Gln	Thr	Arg
CTC	GAG	GAA	GCC	CGG	TCA	GAG	CTC	GCG	GCC
Leu	Glu	Glu	Ala	Arg	Ser	Glu	Leu	Ala	Ala
GC									
	GGC Gly CTC Leu CGG Arg CAG Gln GCG Ala AAC Asn ACC Thr CTG Leu CGG Arg CTC Leu GC	GGC GTG Gly Val CTC TAC Leu Tyr CGG CCG Arg Pro CAG CTG Gln Leu GCG GAC Ala Asp AAC GTC Asn Val ACC TGG Thr Trp CTG CCG Leu Pro CGG GCG Arg Ala CTC GAG Leu Glu GC	GGCGTGACCGlyValThrCTCTACGACLeuTyrAspCGGCCGGTCArgProValCAGCTGGACGlnLeuAspGCGGACCTCAlaAspLeuAACGTCGACThrTrgGlnCTGCCGACCLeuProThrCGGGCGACGArgAlaThrCTCGAGGAALeuGluGluGCSluGlu	GGCGTGACCGTGGlyValThrValCTCTACGACAACLeuTyrAspAsnCGGCCGGTCAGCArgProValSerCAGCTGGACGCGGInLeuAspAlaGCGGACCTCCGGAlaAspLeuArgAACGTCGACGTCAsnValAspGlyACCTGGCAGTGGThrTrpGlnSerCTGCCGACCGGTLeuProThrGlyCTGGAGGAAGCCLeuGluGlaAlaGCSerGAAGCC	GGCGTGACCGTGTCCGlyValThrValSerCTCTACGACAACTTCLeuTyrAspAsnPheCGGCCGGTCAGCCACArgProValSerHisCAGCTGGACGCGCGCGlnLeuAspAlaArgGCGGACCTCCGGCACAlaAspLeuArgHisAACGTCGACGGTCGCAsnValAspGlyArgACCTGGCAGTCGGACThrTrpGlnSerAspCTGCCGACCGGTGTCLeuProThrGlyValCTGGAGGAAGCCCGGLeuGluGluAlaArgLeuGluGluAlaArgGCCCAGCCGCGG	GGCGTGACCGTGTCCCGGGlyValThrValSerArgCTCTACGACAACTTCTTCLeuTyrAspAsnPhePheCGGCCGGTCAGCCACGCCArgProValSerHisAlaCAGCTGGACGCGCGCGTCGInLeuAspAlaArgValGCGGACCTCCGGCACAACAlaAspLeuArgHisAsnAACGTCGACGGTCGCCACAsnValAspGlyArgHisACCTGGCAGGGTGCTCTGThrTrpGlnSerAspGlyCTGCCGACCGGTGTCCTGLeuProThrGlyValLeuCGGGAGGAAGCCCGGTCALeuGluGluAlaArgSerGCGUGAAGCCCGGTCA	GGCGTGACCGTGTCCCGGGAGGlyValThrValSerArgGluCTCTACGACAACTTCTTCCTGLeuTyrAspAsnPhePheLeuCGGCCGGTCAGCCACGCCGAAArgProValSerHisAlaGluCAGCTGGACGCGCGCGTCACGGlnLeuAspAlaArgValThrGCGGACCTCCGGCACAACGGGAlaAspLeuArgHisAsnGlyAACGTCGACGGTCGCCACCACAsnValAspGlyArgHisHisACCTGGCAGGCTGCGGCGGCGThrTrpGlnSerAspGlyValCTGCCGACCGGTGTCCGGGCGAccGCGACGGGTGTCGCGGCGArgAlaThrArgMETValAlaCTGGAGGAAGCCCGGTCAGAGArgAlaThrArgMETValAlaCTGGAGGAAGCCCGGTCAGAGCTGGAGGAAGCCCGGTCAAlaCTGGAGGAAGCCCGGTCAAla </td <td>GGCGTGACCGTGTCCCGGGAGAAGGlyValThrValSerArgGluLysCTCTACGACAACTTCTTCCTGACCLeuTyrAspAsnPhePheLeuThrCGGCCGGTCAGCCACGCCGAACTCArgProValSerHisAlaGluLeuCAGCTGGACGCGCGCGACGAGGInLeuAspAlaArgValThrGluGCGGACCTCCGGCACACCGAGGAGGLAspLeuArgHisAsnGluLeuAACGTCGACGGTCGCCACCACCCGAAAGTCGACGGTCGCCACCACCGGAAAGTCGAGGGTGGTGGTGGTGGTATGAAAGTCGAGGGTGGTGGTGGTGGTGGTATGAAAGTCGAGGAGGGTGGTGGTGGTGGTGGTGGTAAAGTGGAGGGTGGTGGTGGTGGTGGTGGTGGTGGTAAAGTGGAGGGTGGTGGTGGTGGTGGTGGTGGTGGTAAAGTGGGGGGGGGGGGGGGGGGT<td>GGCGTGACCGTGTCCCGGGAGAAGGAGGlyValThrValSerArgGluLysGluCTCTACGACAACTTCTTCCTGACCGTCLeuTyrAspAsnPhePheLeuThrValCGGCCGGTCAGCCACGCCGAACTCGTCArgProValSerHisAlaGluLeuValCAGCTGGACGCGGCCGACACGGAGGTCGInLeuAspAlaArgValThrGluValGCGGACCTCCGGCACAACGGGGTCTACAlaAspLeuArgHisAsnGlyLeuTyrAACGTCGACGGTCGCCACCACCACGCAAAGTCGACGGTGGTGAGGTCTyrAAAGTCGACGGTGACCACCACGCAAAGTCGACGGTGACGACGCGCAAAGTCGACGGTGACGACGACGCAAAGTCGACGGTGACGACGCGCAAAGTCGAGGACGCGGCGACGCAAAGTCGAGGCGGCGAGGCGCAAAGTG</td></td>	GGCGTGACCGTGTCCCGGGAGAAGGlyValThrValSerArgGluLysCTCTACGACAACTTCTTCCTGACCLeuTyrAspAsnPhePheLeuThrCGGCCGGTCAGCCACGCCGAACTCArgProValSerHisAlaGluLeuCAGCTGGACGCGCGCGACGAGGInLeuAspAlaArgValThrGluGCGGACCTCCGGCACACCGAGGAGGLAspLeuArgHisAsnGluLeuAACGTCGACGGTCGCCACCACCCGAAAGTCGACGGTCGCCACCACCGGAAAGTCGAGGGTGGTGGTGGTGGTATGAAAGTCGAGGGTGGTGGTGGTGGTGGTATGAAAGTCGAGGAGGGTGGTGGTGGTGGTGGTGGTAAAGTGGAGGGTGGTGGTGGTGGTGGTGGTGGTGGTAAAGTGGAGGGTGGTGGTGGTGGTGGTGGTGGTGGTAAAGTGGGGGGGGGGGGGGGGGGT <td>GGCGTGACCGTGTCCCGGGAGAAGGAGGlyValThrValSerArgGluLysGluCTCTACGACAACTTCTTCCTGACCGTCLeuTyrAspAsnPhePheLeuThrValCGGCCGGTCAGCCACGCCGAACTCGTCArgProValSerHisAlaGluLeuValCAGCTGGACGCGGCCGACACGGAGGTCGInLeuAspAlaArgValThrGluValGCGGACCTCCGGCACAACGGGGTCTACAlaAspLeuArgHisAsnGlyLeuTyrAACGTCGACGGTCGCCACCACCACGCAAAGTCGACGGTGGTGAGGTCTyrAAAGTCGACGGTGACCACCACGCAAAGTCGACGGTGACGACGCGCAAAGTCGACGGTGACGACGACGCAAAGTCGACGGTGACGACGCGCAAAGTCGAGGACGCGGCGACGCAAAGTCGAGGCGGCGAGGCGCAAAGTG</td>	GGCGTGACCGTGTCCCGGGAGAAGGAGGlyValThrValSerArgGluLysGluCTCTACGACAACTTCTTCCTGACCGTCLeuTyrAspAsnPhePheLeuThrValCGGCCGGTCAGCCACGCCGAACTCGTCArgProValSerHisAlaGluLeuValCAGCTGGACGCGGCCGACACGGAGGTCGInLeuAspAlaArgValThrGluValGCGGACCTCCGGCACAACGGGGTCTACAlaAspLeuArgHisAsnGlyLeuTyrAACGTCGACGGTCGCCACCACCACGCAAAGTCGACGGTGGTGAGGTCTyrAAAGTCGACGGTGACCACCACGCAAAGTCGACGGTGACGACGCGCAAAGTCGACGGTGACGACGACGCAAAGTCGACGGTGACGACGCGCAAAGTCGAGGACGCGGCGACGCAAAGTCGAGGCGGCGAGGCGCAAAGTG



**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.