catalysts

Supplementary Materials: Genetically Fused T4L Acts as a Shield in Covalent Enzyme Immobilisation Enhancing the Rescued Activity

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2.2. Halomonas elongata Aminotransferase

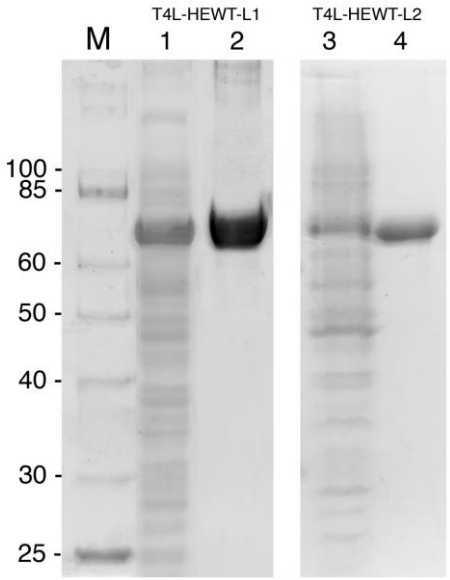

Figure S1. SDS-gel (12%) electrophoresis of T4L-HEWTs. M marker (NEB Broad Range 10-200 kDa); 1 and 2 respectively the soluble crude fraction and the purified T4L-HEWT_L1; 3 and 4 respectively the soluble crude fraction and the purified T4L-HEWT_L2.

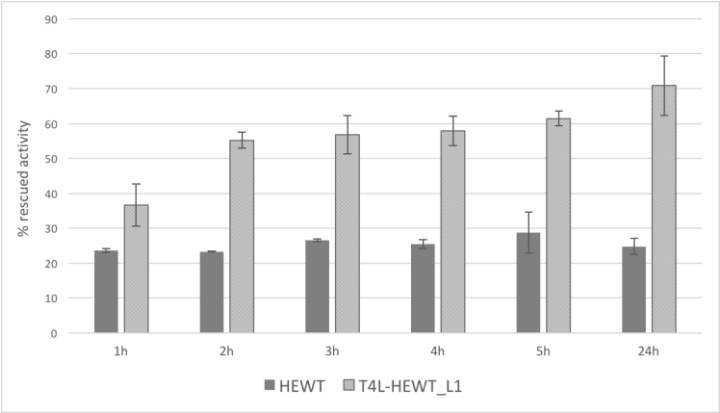

Figure S2. HEWTs immobilisation at different incubation times (room temperature, in 50 mM phosphate buffer pH 8). HEWT (dark columns), and T4L+HEWT_L1 (light columns), immobilisation varying the time of contact between the enzyme and the solid support (Sepabeads EC-EP/S (pore ø 10-20 nm)). Immobilisation performed using a 5 mg_{enzyme}/g_{resin}.

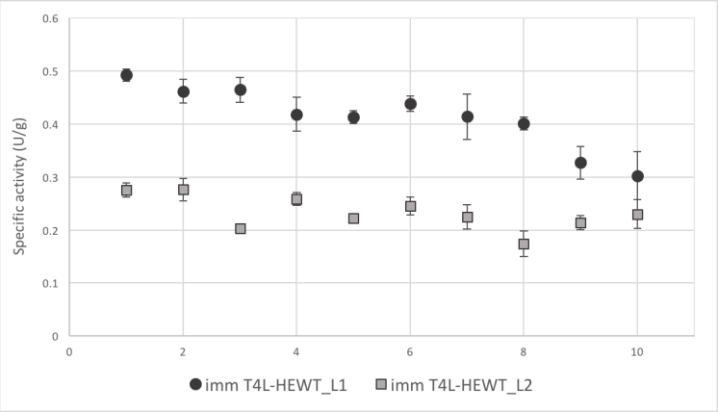

Figure S3. Reusability profile of the immobilised T4L-HEWT_L1 (circle) and T4L-HEWT_L2 (square) after ten reaction cycles. The experiment was conducted repeating the activity assay ten times; every time the imm-HEWT was isolated from the exhausted mixture and used in the following run. In this assay, a resin Sepabeads EC-EP/S (pore ø 10-20 nm) loaded with 1 mg_{enzyme}/g_{resin} was used.

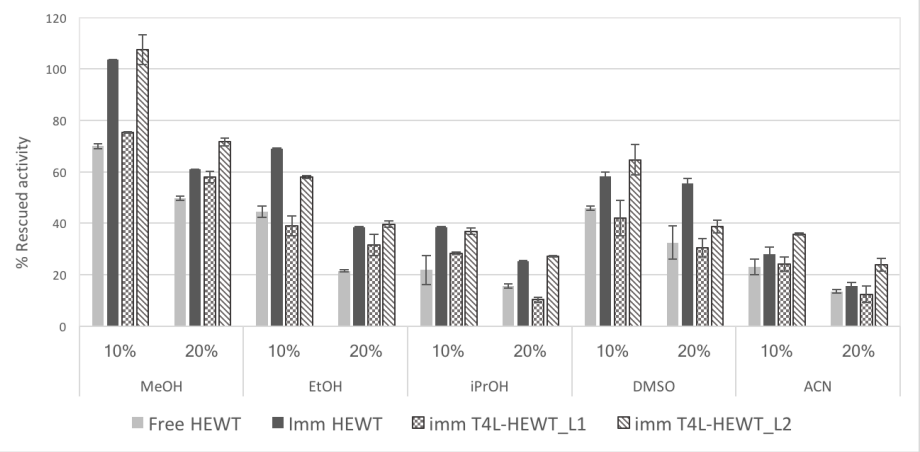

Figure S4. Stability of HEWTs in different organic co-solvents. HEWT (light grey columns), imm-HEWT (dark grey columns), imm-T4L+HEWT_L1 (chess columns), and imm-T4L+HEWT_L2 (dash columns) at 10 and 20% co-solvent concentration in 50 mM phosphate, pH 8.0 buffer, after 24 hours incubation at 4 °C. In this assay, a resin Sepabeads EC-EP/S (pore ø 10-20 nm) loaded with 1 mg_{enzyme}/g_{resin} was used.

2.3. Bacillus subtilis Esterase

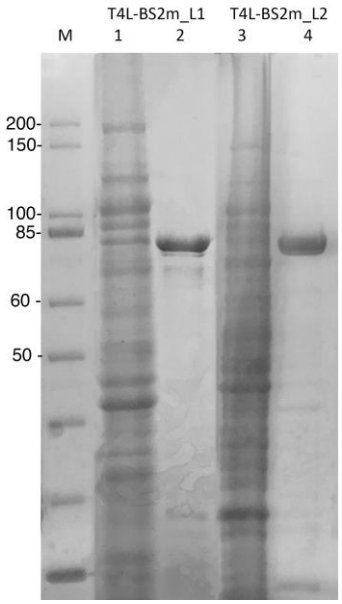

Figure S5. SDS-gel (12%) electrophoresis of T4L-BS2ms. M marker (NEB Broad Range 10-200 kDa); 1 and 2 respectively the soluble crude fraction and the purified T4L-BS2m_L1; 3 and 4 respectively the soluble crude fraction and the purified T4L-BS2m_L2.

2.4. Horse Liver Alcohol Dehydrogenase

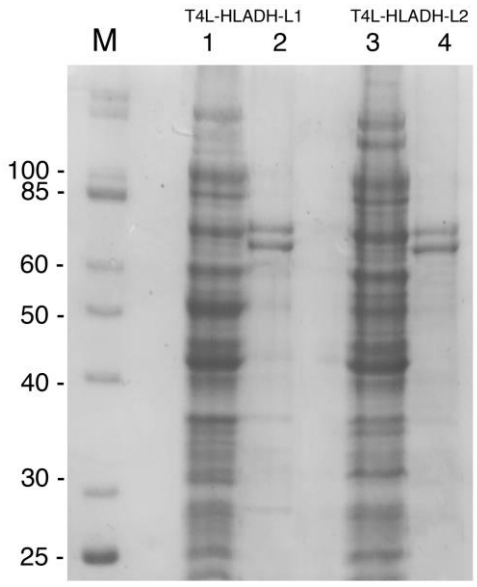

Figure S6. SDS-gel (12%) electrophoresis of T4L-HLADHs. M marker (NEB Broad Range 10-200 kDa); 1 and 2 respectively the soluble crude fraction and the his-tag purified of T4L-HLADH_L1; 3 and 4 respectively the soluble crude fraction and the his-tag purified of T4L-HLADH_L2.

4. Materials and Methods

4.1. T4L- HEWT, T4L-BS2m, and T4L-HLADH Constructs Generation

T4L-HEWT_L1:

MRGSHHHHHHGMASMTGGQQMGRDENLYFQGDP*NIFEMLRID**Q**GLRLKIYKDTEGYYTIGIGHLLTKSPSL*
NAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDAAVRGILRNAKLKPVYDSLDAVRRAALINMVFQMGETGVA
*GFTNSLRMLQQKRWDEAAVNLAKSRWYNQTPNRAKRVITTFRTGTWDAY***LEICSWYHG***MQTQDYQALDRA*
HHLHPFTDFKALGEEGSRVVTHAEGVYIHDSEGNRILDGMAGLWCVNLGYGRRELVEAATAQLEQLPYYNTFFK
TTHPPAVRLAEKLCDLAPAHINRVFFTGSGSEANDTVLRMVRRYWALKGQPDKQWIIGRENAYHGSTLAGMSL
GGMAPMHAQGGPCVPGIAHIRQPYWFGEGRDMSPEAFGQTCAEALEEKILELGEEKVAAFIAEPVQGAGGAIM
PPESYWPAVKKVLAKYDILLVADEVICGFGRLGEWFGSQHYGLEPDLMPIAKGLSSGYLPIGGVLVGDRVAETLIE
EGGEFFHGFTYSGHPTCAAVALKNLELLEAEGVVDRVRDDLGPYLAERWASLVDHPIVGEARSLGLMGALELVA
DKTTGQRFDKSLGAGNLCRDLCFANGLVMRSVGDTMIISPPLVIRREEIDELVELARRALDETARQLTQVPHTQE
EPTA

T4L-HEWT_L2:

MRGSHHHHHHGMASMTGGQQMGRDENLYFQGDP*NIFEMLRID**Q**GLRLKIYKDTEGYYTIGIGHLLTKSPSL*
NAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDAAVRGILRNAKLKPVYDSLDAVRRAALINMVFQMGETGVA
*GFTNSLRMLQQKRWDEAAVNLAKSRWYNQTPNRAKRVITTFRTGTWDAY***LHG***MQTQDYQALDRAHHLHPF*
TDFKALGEEGSRVVTHAEGVYIHDSEGNRILDGMAGLWCVNLGYGRRELVEAATAQLEQLPYYNTFFKTTHPPA
VRLAEKLCDLAPAHINRVFFTGSGSEANDTVLRMVRRYWALKGQPDKQWIIGRENAYHGSTLAGMSLGGMAP
MHAQGGPCVPGIAHIRQPYWFGEGRDMSPEAFGQTCAEALEEKILELGEEKVAAFIAEPVQGAGGAIMPPESYW
PAVKKVLAKYDILLVADEVICGFGRLGEWFGSQHYGLEPDLMPIAKGLSSGYLPIGGVLVGDRVAETLIEEGGEFF
HGFTYSGHPTCAAVALKNLELLEAEGVVDRVRDDLGPYLAERWASLVDHPIVGEARSLGLMGALELVADKTTG
QRFDKSLGAGNLCRDLCFANGLVMRSVGDTMIISPPLVIRREEIDELVELARRALDETARQLTQVPHTQEEPTA

T4L-BS2m_L1:

*MRGSHHHHHHGMASMTGGQQMGRDENLYFQGDP*NIFEMLRID***Q***GLRLKIYKDTEGYYTIGIGHLLTKSPSL*
NAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDAAVRGILRNAKLKPVYDSLDAVRRAALINMVFQMGET
GVAGFTNSLRMLQQKRWDEAAVNLAKSRWYNQTPNRAKRVITTFRTGTWDAY**LEICSWYHG***MTHQIVTT*
QYGKVKGTTENGVHKWKGIPYAKPPVGQWRFKAPEPPEVWEDVLDATAYGSICPQPSDLLSLSYTELPRQSEDC
LYVNVFAPDTPSKNLPVMVWIHGGAFYLGAGSEPLYDGSKLAAQGEVIVVTLNYRLGPFGFLHLSSFNEAYSDNL
GLLDQAAALKWVRENISAFGGDPDNVTVFGESAGGMSIAALLAMPAAKGLFQKAIMESGASRTMTKEQAASTS
AAFLQVLGINEGQLDKLHTVSAEDLLKAADQLRIAEKENFFQLFFQPALDPKTLREEPEKAIAEGAASGIPLLIGTT
RDEGYLYFTPDSDVHSQETLDAALEYLLGKPLAEKVADLYPRSLESQIHMMTDLLFWSPAVAYASAQSHYAPV
WMYRFDWHPKKPPYNKAFHALELPFVFGNLDGLERMAKAEITDEVKQLSHTIQSAWITFAKTGNPSTEAVNWP
AYHEETRETLILDSEITIENDPESEKRQKLFPSKGEGS

T4L-BS2m_L2:

*MRGSHHHHHHGMASMTGGQQMGRDENLYFQGDP*NIFEMLRID***Q***GLRLKIYKDTEGYYTIGIGHLLTKSPSL*
NAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDAAVRGILRNAKLKPVYDSLDAVRRAALINMVFQMGET
GVAGFTNSLRMLQQKRWDEAAVNLAKSRWYNQTPNRAKRVITTFRTGTWDAY**LHG***MTHQIVTTQYGKVK*
GTTENGVHKWKGIPYAKPPVGQWRFKAPEPPEVWEDVLDATAYGSICPQPSDLLSLSYTELPRQSEDCLYVNVF
APDTPSKNLPVMVWIHGGAFYLGAGSEPLYDGSKLAAQGEVIVVTLNYRLGPFGFLHLSSFNEAYSDNLGLLDQ
AAALKWVRENISAFGGDPDNVTVFGESAGGMSIAALLAMPAAKGLFQKAIMESGASRTMTKEQAASTSAAFLQ

VLGINEGQLDKLHTVSAEDLLKAADQLRIAEKENFFQLFFQPALDPKTLREEPEKAIAEGAASGIPLLIGTTRDEGY
LYFTPDSDVHSQETLDAALEYLLGKPLAEKVADLYPRSLESQIHMMTDLLFWSPAVAYASAQSHYAPVWMYRF
DWHPKKPPYNKAFHALELPFVFGNLDGLERMAKAEITDEVKQLSHTIQSAWITFAKTGNPSTEAVNWPAYHEET
RETLILDSEITIENDPESEKRQKLFPSKGEGS

T4L-HLADH_L1:

MRGS<u>*HHHHHH*</u>*GMASMTGGQQMGRDENLYFQGDP*NIFEMLRID**Q**GLRLKIYKDTEGYYTIGIGHLLTKSPSL
NAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDAAVRGILRNAKLKPVYDSLDAVRRAALINMVFQMGET
GVAGFTNSLRMLQQKRWDEAAVNLAKSRWYNQTPNRAKRVITTFRTGTWDAY**LEICSWYHG***MSTAGKVI*
KCKAAVLWEEKKPFSIEEVEVAPPKAHEVRIKMVATGICRSDDHVVSGTLVTPLPVIAGHEAAGIVESIGEGVTTV
RPGDKVIPLFTPQCGKCRVCKHPEGNFCLKNDLSMPRGTMQDGTSRFTCRGKPIHHFLGTSTFSQYTVVDEISVA
KIDAASPLEKVCLIGCGFSTGYGSAVKVAKVTQGSTCAVFGLGGVGLSVIMGCKAAGAARIIGVDINKDKFAKAKE
VGATECVNPQDYKKPIQEVLTEMSNGGVDFSFEVIGRLDTMVTALSCCQEAYGVSVIVGVPPDSQNLSMNPMLL
LSGRTWKGAIFGGFKSKDSVPKLVADFMAKKFALDPLITHVLPFEKINEGFDLLRSGESIRTILTF

T4L-HLADH_L2:

MRGS<u>*HHHHHH*</u>*GMASMTGGQQMGRDENLYFQGDP*NIFEMLRID**Q**GLRLKIYKDTEGYYTIGIGHLLTKSPSL
NAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDAAVRGILRNAKLKPVYDSLDAVRRAALINMVFQMGET
GVAGFTNSLRMLQQKRWDEAAVNLAKSRWYNQTPNRAKRVITTFRTGTWDAY**LHG***MSTAGKVIKCKAAV*
LWEEKKPFSIEEVEVAPPKAHEVRIKMVATGICRSDDHVVSGTLVTPLPVIAGHEAAGIVESIGEGVTTVRPGDKVI
PLFTPQCGKCRVCKHPEGNFCLKNDLSMPRGTMQDGTSRFTCRGKPIHHFLGTSTFSQYTVVDEISVAKIDAASP
LEKVCLIGCGFSTGYGSAVKVAKVTQGSTCAVFGLGGVGLSVIMGCKAAGAARIIGVDINKDKFAKAKEVGATEC
VNPQDYKKPIQEVLTEMSNGGVDFSFEVIGRLDTMVTALSCCQEAYGVSVIVGVPPDSQNLSMNPMLLLSGRTW
KGAIFGGFKSKDSVPKLVADFMAKKFALDPLITHVLPFEKINEGFDLLRSGESIRTILTF

4.2. Expression, Purification, and Characterization of the HEWT, BS2m, HLADH, and T4L Proteins in E. coli

Table S1: computed molecular weight (MW) and molar extinction coefficients (ε) [1].

Protein	MW (kDa)	ε (mM^{-1} cm^{-1})
HEWT	54.2	61.4
T4L-HEWT_L1	73.3	93.8
T4L-HEWT_L2	72.5	86.7
BS2m	55.0	81.9
T4L-BS2m_L1	77.1	115.9
T4L-BS2m_L2	76.3	108.9
HLADH	43.8	19.3
T4L-HLADH_L1	62.8	51.7
T4L-HLADH_L2	62.0	44.7
T4L	25.8	39.4

Reference:

[1] Gasteiger E., Hoogland C., Gattiker A., Duvaud S., Wilkins M.R., Appel R.D., Bairoch A. Protein Identification and Analysis Tools on the ExPASy Server. John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press **2005**, 571-607.