Supporting Information

Synthesis Of A Heterogeneous Artificial Metallolipase with Chimeric Catalytic Activity.

Marco Filice,* Oscar Romero, Javier Gutiérrez-Fernández, Blanca de las Rivas, Juan A. Hermoso, José M. Palomo.

*E-mail: marco.filice1@gmail.com

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Experimental Section

Materials

CNBr-activated Sepharose®, Butyl-Sepharose® 4 Fast Flow and Sepharose 4BCL were from GE Healthcare (Uppsala, Sweden). Sepabeads® epoxyde (EC-EP) and Sepabeads® butyl (EC-BU) were kindly provided by Resindion (Mitsubishi Chemical Corporation, Milan, Italy). Benzamidine, Dithiothreitol (DTT), 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB), sodium borohydride (NaBH₄), Triton® X-100, Iodoacetic acid, Copper (II) nitrate trihydrate and p-nitrophenylbutyrate (pNPB) were from Sigma Aldrich. 1,10-Phenanthrolin-5-amine was from Toronto Research Chemicals (Canada). All the other reagents were of analytical grade. The spectrophotometric analyses were run on a V-630 spectrophotometer (JASCO, Japan). HPLC spectrum P100 (Thermo Separation products) was used. Analyses were run at 25°C using an L-7300 column oven and a UV6000LP detector. MALDI-TOF analyses were carried out with a Bruker MicroFlex MALDI-TOF (Bruker Daltonics).

Methods

Site-directed mutagenesis, cloning and expression of GTL mutants.

All site-directed mutagenesis experiments were carried out by PCR using mutagenic primers. Briefly, to introduce the amino acid change, the corresponding pair of primers was used as homologous primer pair in a PCR reaction using a specific plasmid as template and Prime Start HS Takara DNA polymerase. The product of the PCR was digested with endonuclease DpnI that exclusively restricts methylated DNA. *E. coli* DH10B cells were transformed directly with the digested product. The plasmid with mutated lipase were identified by sequencing and then transformed into *E. coli* BL21 (DE3) cells to express the corresponding proteins. Firstly, a double mutant without cysteines on its structure was created (GTL*). To this scope, the initial C65S mutant was created and the resulting

plasmid was used as template to create the double mutant C65S/C296S-GTL (no cysteines present in the enzyme structure). Hence, this plasmid was used as template to construct additional mutations (S114C and S196C) using the different mutagenic primers reported in the following Table.

The gene corresponding to the mature lipase from *G. thermocatenulatus* (GTL) was cloned into pT1 expression vector as previously described.[1] Cells carrying the recombinant plasmid pT1BTL2 were grown at 30°C and over expression was induced by raising the temperature to 42°C for 20 h. Then, the cells were harvested by centrifugation and washed with distilled water. Finally, the cells were disrupted by sonication and centrifuged. The supernatant was stored at -80°C for further purification.

Primers used for site-directed mutagenesis of GTL.

Mutant [a]	Plasmid template	Primers [b]
GTL-C65S	pT1GTL	Cys/Ser 65-5 5´-CAACTGGGACCGGGCGagcGAAGCGTACGCCCAG´ Cys/Ser 65-3 5´-CTGGGCGTACGCTTCgctCGCCCGGTCCCAGTTG´
GTL-C65S/C296S (GTL*)	pT1GTL2mutC65S	Cys/Ser 296-5 5´-GCGGTCGTAagcGCCCCGTTTC Cys/Ser 296-3 5´-GAAACGGGGCgctTACGACCGC
C65S/C296S/S114C (GTL*/114)	pT1GTL2mutCys[c]	Ser/Cys 114-5 5' CATCGCTCATtgcCAAGGAGGAC Ser/Cys 114-3 5' GTCCTCCTTGgcaATGAGCGATG
C65S/C296S/S196C (GTL*/196)	pT1GTL2mutCys[c]	Ser/Cys196-5 5´-GTTGAAAGCGGCGGCTGTCGCCtgcAATGTGCCGTACACGAGTCAAG´ Ser/Cys196-3 5´-CTTGACTCGTGTACGGCACATTgcaGGCGACAGCCGCCGCTTTCAAC

[a] The mutant name shows the amino acid changes and its position in GTL.

[b] The nucleotide changes used to introduce the mutation are indicated in lower case

[c] Plasmid with GTL mutant lacking both of the two native Cys residues (Cys65 and Cys 296).

Purification and immobilization on SP-C$_4$ of GTL variants

The enzyme was purified from *Escherichia coli* crude extract by interfacial adsorption on Butyl-Sepharose (SP-C$_4$).[2] Briefly, 4 mL of the crude extract were diluted with 16 mL of 10 mM sodium phosphate and 3 mM of Benzamidine at pH 7. To this solution, 1 g of SP-C$_4$ support was added and the resulting suspension was kept on mild stirring for 30 min. Finally, the immobilized enzyme was washed with abundant distilled water and analyzed by SDS-PAGE (Figure S2).

The immobilization yields and lipase loading amount were calculated by Bradford assay in the case of GTL*/114 mutant and by Bradford and lipase activity assays in the case of GTL*/196 mutant.

Enzymatic activity assay

The activities of all the soluble and immobilized GTL variants of were analyzed spectrophotometrically measuring the increment in absorbance at 348 nm (isosbestic point) produced by the release of p-nitrophenol (pNP) (ε= 5,150 M-1 cm^{-1}) in the hydrolysis of 0.4 mM pNPB in 25 mM sodium phosphate at pH 7 and 25 °C. To initialize the reaction, 0.05–0.2 mL of lipase solution or suspension was added to 2.5 mL of substrate solution. Enzymatic activity is given as micromole of hydrolyzed pNPB per minute per milligram of enzyme (IU) under the conditions above described.

Synthesis of the Ligand 1

The phenantroline-based ligand **1** was synthesized as reported in literature.[3]

Solid-phase synthesis of the artificial metallolipase

1 g of the GTL variants immobilized on SP-C$_4$ was incubated in 10 mL of DTT solution (50 mM in 25 mM sodium phosphate at pH 8) for 30 min. After that, the reduced biocatalysts were thoroughly washed with distilled water and immediately used. To promote the site-selective modification of reduced cysteine, 1 g of SP-C$_4$-GTL* variants (generally loaded with 10 mg of pure lipase mutant) were resuspended in 10 mL of 25 mM sodium phosphate buffer containing 5 % (v/v) of DMSO and 10 eq. of **1** (respect to enzyme moles) previously dissolved in a small amount of DMSO. The pH value was then set to 8 and the reaction was kept under mild agitation and protected from light for overnight. Subsequently, the modified immobilized enzyme was washed with water and 5 % (v/v) of DMSO (3 x 10 mL) and abundant distilled water. The coupling efficiency was determined by Ellman's assay as described below. Then, to incorporate the Cu^{2+} catalytic metal, 1 g of the SP-C$_4$-GTL*/Ψ conjugates were incubated in 10 mL of an aqueous solution containing 10 eq. of Cu(NO$_3$)$_2$*3H$_2$O (respect to enzyme moles) for 1 h. Finally the immobilized enzymes were washed with abundant distilled water.

In order to assess the possibility of unspecific adsorption of Cu^{2+} cation to GTL* structure, the entire strategy was performed using each reported mutant but without carrying out the modification with phenanthroline-based ligand **1**.

Determination of coupling efficiency

The coupling efficiency was determined by comparison among the free thiols titrated by Ellman's assay before and after the conjugation of ligand **1** to each reported GTL mutant.[4]. Briefly, to 25-50 mg of the immobilized lipase immobilized on SP-C$_4$ 1 mL of a mixture of 1.5 mM of DTNB in 250 mM sodium phosphate buffer pH 8.0 was added. After 30 min, the

absorbance of the supernatant was measured at 412 nm. Using the extinction coefficient (ε_{412} = 13600 M^{-1} cm^{-1}) the amount of free thiols was determined.

Oriented Immobilization of the artificial metallolipase.

The artificial enzyme immobilized on SP-C$_4$ was desorbed from the support adding 20 mL of 25 mM phosphate buffer pH 7 with 0.5% Triton X-100 (v/v) per gram of support and keeping under mild stirring for 30 min. The suspension was filtered, and the supernatant containing the pure soluble artificial metallolipase was used for the further immobilization on different supports (Figure S5; Table S1)

One point covalent attachment (SP-εNH$_2$ and SB-εNH$_2$):

The immobilization of GTL*/Ψ on commercial CNBr-activated Sepharose (SP-εNH$_2$) was carried out as reported by the manufacturer. The only modification is related to the incubation time that it is reduced to max 30 min before to block the immobilization reaction (in order to ensure the one point immobilization *via* terminal amino).

When the aldehyde-activated Sepabeads support was used, the immobilization reaction was carried out as previously described.[5] Briefly, to 10 mL of the enzymatic solution of soluble GTL artificial catalyst obtained as above described, 50 mM of DTT and 100 mM of sodium bicarbonate were added and the pH was brought to 8. After that, 1 g of the aldehyde support SB-CHO was added and resulting suspension was maintained under gently stirring at RT for 12 h. Finally, in order to reduce the imino and aldehyde groups and stop the immobilization reaction, 10 mg sodium borohydride were added under gentle stirring. After 30 min, the immobilized enzyme was washed with abundant distilled water.

Multipoint covalent attachment (SP-Lys and SB-Lys):

To 10 mL of the enzymatic solution of soluble GTL artificial catalyst obtained as above described, 100 mM of sodium bicarbonate were added and the pH was brought to 10.1. After that, 1 g of the aldehyde supports (SP-CHO or SB-CHO) was added and the resulting suspension was maintained under gently stirring at RT for 12 h. Finally, in order to reduce the imino and aldehyde groups and stop the immobilization reaction, 10 mg sodium borohydride were added under gentle stirring. After 30 min, the immobilized enzyme was washed with abundant distilled water.

In both cases, the immobilization yields were calculated by Bradford assay in the case of GTL*/114 mutant and by lipase activity assay in the case of GTL*/196 mutant.

MALDI-TOF-MS spectra

The GTL variants (mutants and conjugate derivatives) were desorbed with Triton X-100 from SP-C_4 support and desalted using Ziptip C18 (Merck) following the manufacturer's instructions. After that, 1 µL of sample dissolved in 50% v/v ACN with 0.1% TFA was spotted on the MALDI target and mixing with 1 µL of saturated solution of sinapinic acid matrix (dissolved in 30% v/v of ACN with 0.1% TFA). The samples were analyzed with a ultrafleXtreme MALDI TOF/TOF mass spectrometer (Bruker) (Figure S3).

Trypin digestion of GTL variants

The GTL variants (mutants and conjugate derivatives) were desorbed with Triton X-100 from SP-C_4 support. After that, 10 µL of the enzyme solution was diluted with 10 µL of 100mM TrisHCl pH 8.0, reduced with dithiothreitol (DTT), alkylated with iodoacetamide

(IAA) and incubated for 24 h with Trypsin (mass spectrometry grade, ratio 1:20) at 37°C. The digestion was stopped by addition of 30 µL of acetonitrile and the samples were dried in vacuum concentrator. Afterwards the peptides were dissolved in 0.3% of TFA and desalted using Ziptip C18 (Merck) following the manufacturer's instructions. After that, 1 µL of sample dissolved in 50% v/v ACN with 0.1% TFA was spotted on the MALDI target and mixing with 1 µL of saturated solution of α-cyano-4-hydroycinnamic acid (dissolved in 50% v/v of ACN with 0.1% TFA). The samples were analyzed with an ultrafleXtreme MALDI TOF/TOF mass spectrometer (Bruker). The peptides were identified using the Biotools Software 3.2 (Bruker) and the previously reported sequence of GTL.[10] The sequence coverage obtained fluctuates between 45-55% (Figure S4).

Diels Alder reactions

The azachalcone reactant **2** was prepared as reported in literature.[6] The Diels Alder products **4** and **5** as reference compounds were prepared, purified and characterized as described in literature.[6]

The Diels Alder reaction used for the assessment of each heterogeneous artificial metallolipase was carried out as follow. To 1 mL of 10 mM MES buffer/ACN 95/5 pH 6, 10 µl of a 100 mM azachalcone **2** solution in ACN were added (final concentration 1 mM). After that, the resulting solution was cooled to 4°C, preserved by light and 100 mg of each immobilized catalyst (generally 1 mg of each metallolipase, 0.238% mol respect to **2**) was added under mechanical stirring. Immediately, in order to initialize the reaction, 8 µl of freshly distilled cyclopentadiene were added (final concentration 10 mM) and kept on mechanical stirring (orbital shaker) at 4°C for 72h. To analyze the reaction in HPLC, the supernatant was recovered by filtration and extracted with Et_2O (3 x 1 mL). Subsequently,

the organic layer was dried over Na_2SO_4, filtered and evaporated. The oily glassy residue was dissolved in the analysis mobile phase. The *ee* of each reaction was determined on a Lux-Cellulose 1 column (250x4.6 μm; Ø 3 μm) (Phenomenex) with n-hexane/2-propanol = 99/1, flow = 1 mL/min, λ= 265 nm (Figure S6). This analytical method was successfully validated by positive comparison with the standard analytical methods described in literature.[6,7,8] All of the experiments were performed in triplicate, and the experimental error was less than 5%.

Scaled-up Diels Alder reaction for stability study

To 4.75 mL of 10 mM MES buffer/ACN 50/50 (to ensure the full solubility of reactants) pH 6, 250 μl of a 100 mM azachalcone **2** in ACN solution were added (final concentration 5 mM). After that, the solution was cooled to 4°C, preserved by light and 500 mg of SB-Lys-GTL*/196-Ψ-Cu^{2+} artificial metallolipase (generally 5 mg of each metallolipase) were added under stirring. Immediately, in order to initialize the reaction, 200 μl of freshly distilled cyclopentadiene were added (final concentration 50 mM) and kept on mechanical stirring (orbital shaker) at 4°C for 72h. The HPLC reaction work up and analysis was carried out as previously described

General procedure for Diels-Alder recycling studies

The SB-Lys-GTL*/196-Ψ-Cu^{2+} artificial metallolipase was recovered after each reaction cycle by filtration and then washed with 3 x 1 mL of water, 3 x1 mL of methanol and 3 x 1 mL of Et_2O. The obtained solid was dried under reduced pressure and reused for next reaction cycle maintaining the same reaction ratios.

Domino synthesis of 4-aminophenol (8).

To 2.25 ml of 25 mM sodium phosphate pH 7 buffer, 0.25 mL of 100 mM p-nitrophenyl butyrate **6** solution in ACN (final concentration 10 mM) was added under magnetic stirring at 25 °C and let to homogenize. After that, 100 mg of SB-Lys-GTL*/196-Ψ-Cu^{2+} artificial metallolipase were added. The reaction was kept on gentle stirring until complete conversion of substrate **6** to product **7** that it was achieved about after 40 min (Figure S12). Subsequently, to initialize the Cu^{2+}-catalyzed reduction of **7** to **8**, 37.8 mg of solid NaBH$_4$ (0.001 moles) were directly added to the reaction mixture. The reaction was kept on gentle stirring at 25 °C until complete conversion of substrate **7** to product **8** that it was achieved about after 1h (Figure S12). Both the reaction progresses (enzymatic as well as reduction) were monitored by taking out 20 μl of the reaction solution, diluting it with 2 mL of distilled water and measuring the absorption spectrum between 600 and 200 nm in a quartz cuvette.

Docking calculations.

Three-dimensional structures of different ligands used during docking calculations were built in PyMol (The PyMOL Molecular Graphics System, Version 1.7.2 Schrödinger, LLC) and their stereochemistry regularized with phenix_elbow, from the Phenix suite.[9] The crystal structure of the catalytically active open state of the GTL lipase (PDB code 2W22)[10] was used to dock ligand **1** at sites 114 and 196 by using GOLD (Genetic Optimization for Ligand Docking)[11] by defining a partially flexible cavity of 10 Å around the cysteine to which Ψ–Cu^{2+} is covalently bound. For each of the 25 independent genetic algorithm runs, a maximum number of 100,000 operations were performed on a set of five groups with a

population size of 100 individuals. Default cut-off values of 2.5 Å (dH-X) for hydrogen bonds and 4.0 Å for Van der Waals distance were employed. When the top three solutions attained RMSD values within 1.5 Å, docking was terminated. The RMSD values for the docking calculations are based on the RMSD matrix of the ranked solutions. Best-ranked solutions were always among the first 10 genetic algorithm runs, and the conformation of molecules based on the best fitness score was further analyzed. After having obtained good docking solutions for ligand $\Psi-Cu^{2+}$ in both sites, additional dockings were performed using the four possible products of the reaction (**4a**, **4b**, **5a** and **5b**) as flexible ligands, in order to predict their orientation in each pocket before the release to the solvent. Side chains inside a sphere of 10 Å centered on the Cu^{2+} atom were considered to be flexible during these dockings. The same docking strategy used with ligand **1** was applied in these new cases. The docking poses are ranked based on a molecular mechanics–like scoring function, which includes a hydrogen-bond term, an intermolecular van der Waals term, and an intramolecular van der Waals term for the internal energy of the ligand and ligand torsional strain energy. Best-ranked solutions were always among the first 10 genetic algorithm runs, and the conformation of molecules based on the best fitness score was further analyzed.

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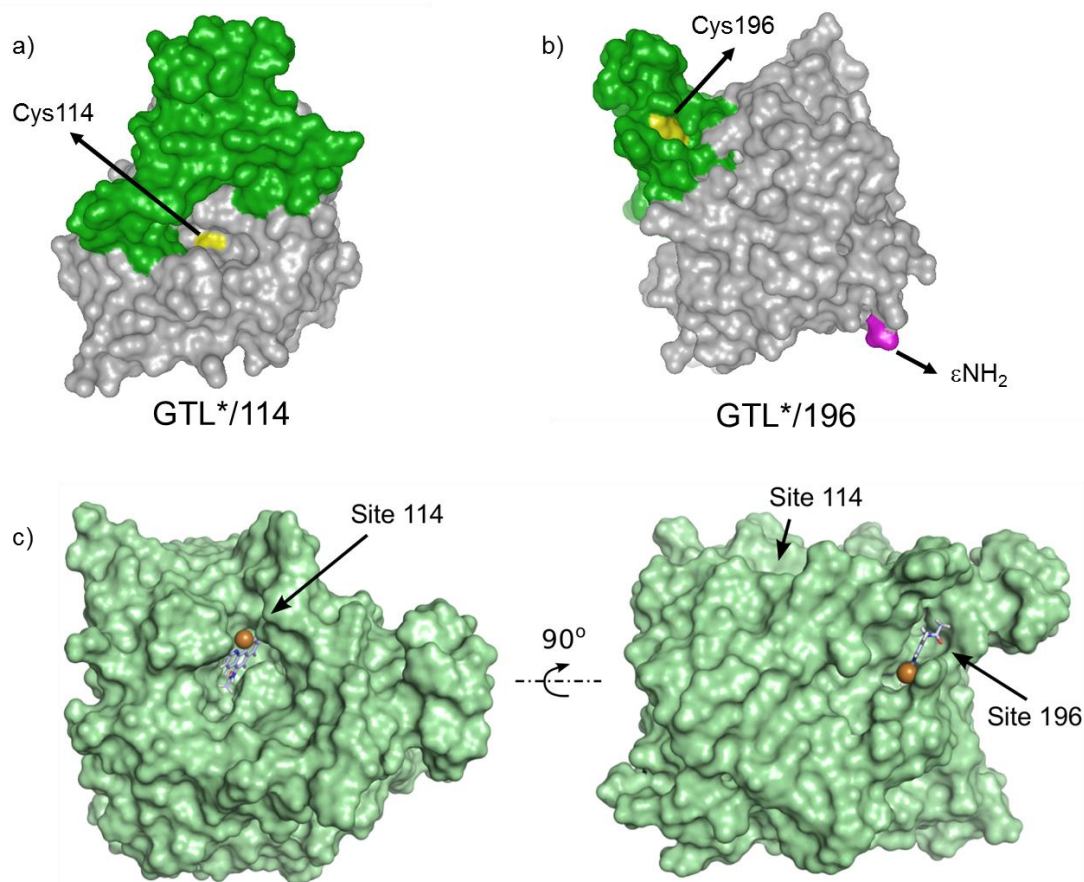(11) M. L. Verdonk, J. C. Cole, M. J. Hartshorn, C. W. Murray and R. D. Taylor, *Proteins*, 2003, **52**, 609.

Figure S1. a) GTL triple mutant with unique cysteinyl group located at position 114 (native catalytic site). b) GTL triple mutant with unique cysteinyl group located at position 196 (β-winged helix turn-helix domain present into the enzyme's lid). Surface color codes: green: GTL's lid residues; purple: terminal amino group; yellow: mutated residues. c) Overall view of ψ-Cu^{2+} complex insertion on different position of GTL* structure.
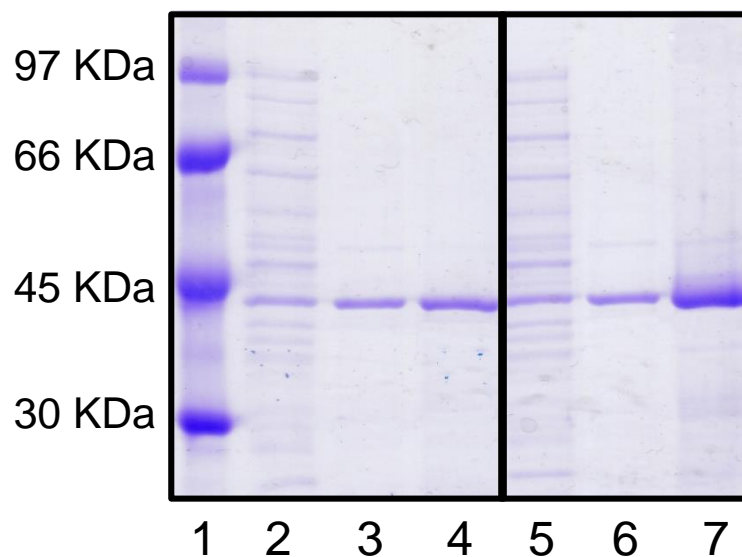
Figure S2. SDS-PAGE of purification, immobilization and site-directed modification of GTL* mutants. 1) Low Molecular Weights; 2) Crude extract of GTL*/114 mutant; 3) SP-C$_4$-GTL*/114 derivative; 4) SP-C$_4$-GTL*/114-Ψ derivative; 5) Crude extract of GTL*/196 mutant; 6) SP-C$_4$-GTL*/196 derivative; 7) SP-C$_4$-GTL*/196-Ψ derivative.
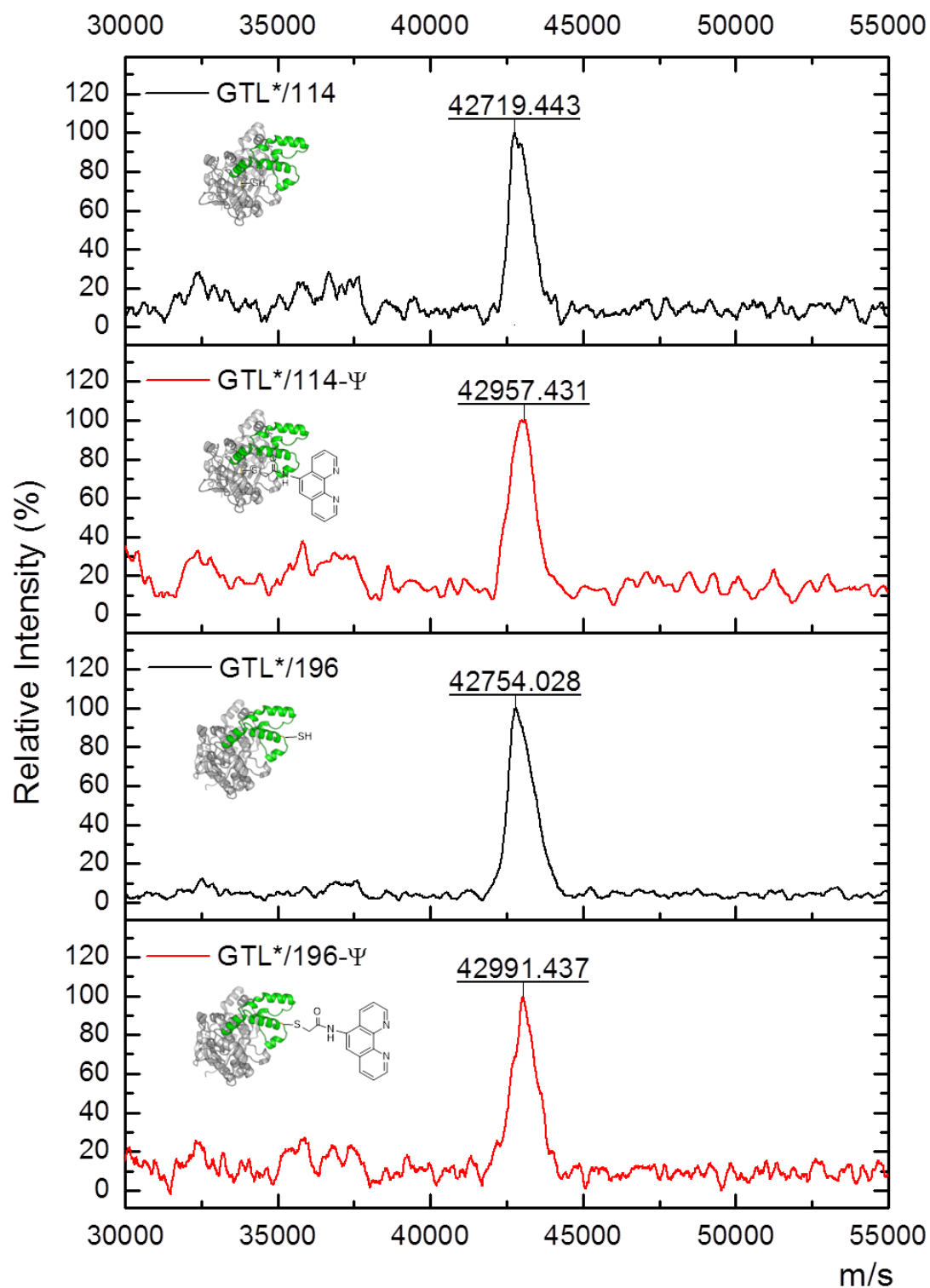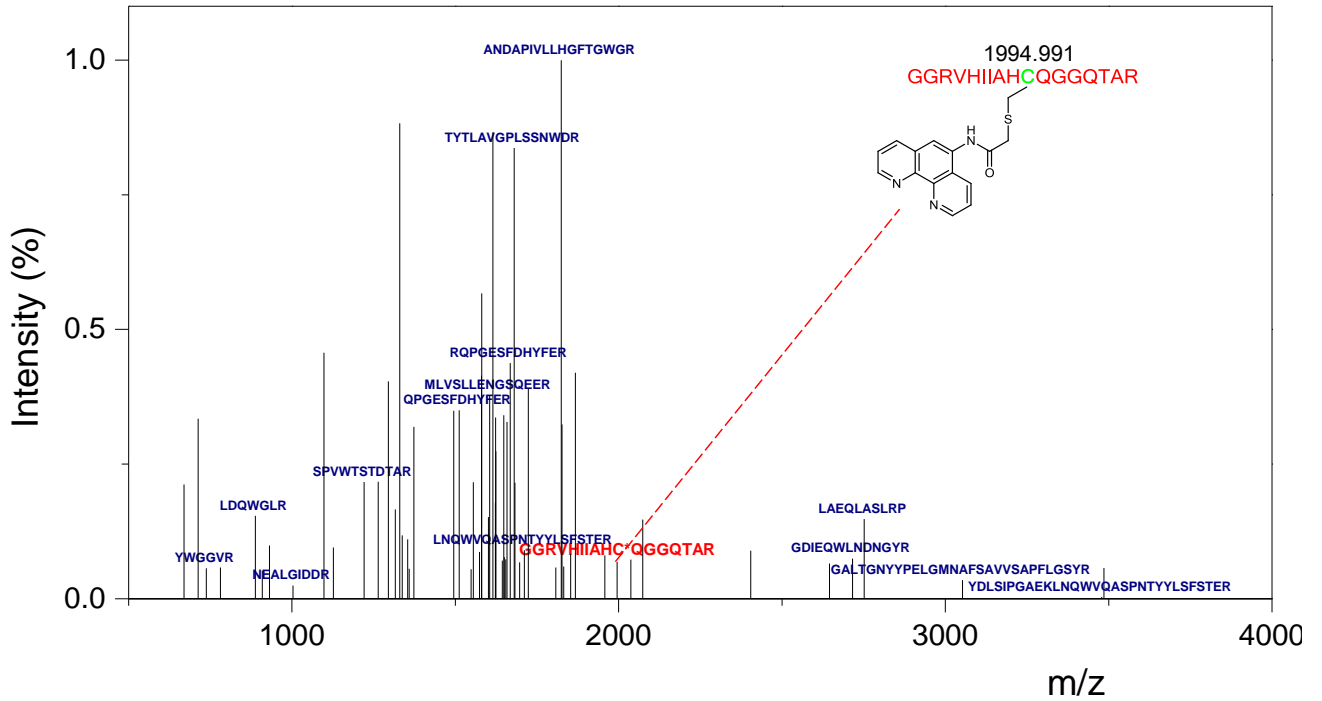
Figure S3. MALDI traces of GTL mutants before and after modification with phenantroline based ligand **1** (MW: 236.26).

Figure S4. Tryptic digestion and sequence analysis of GTL*/114-Ψ .

	a	Cys196	b	Cys196
Lys			Cys114	N-Terminal
Cys114				

⭐ Cysteine 114   ⭐ Cysteine 196

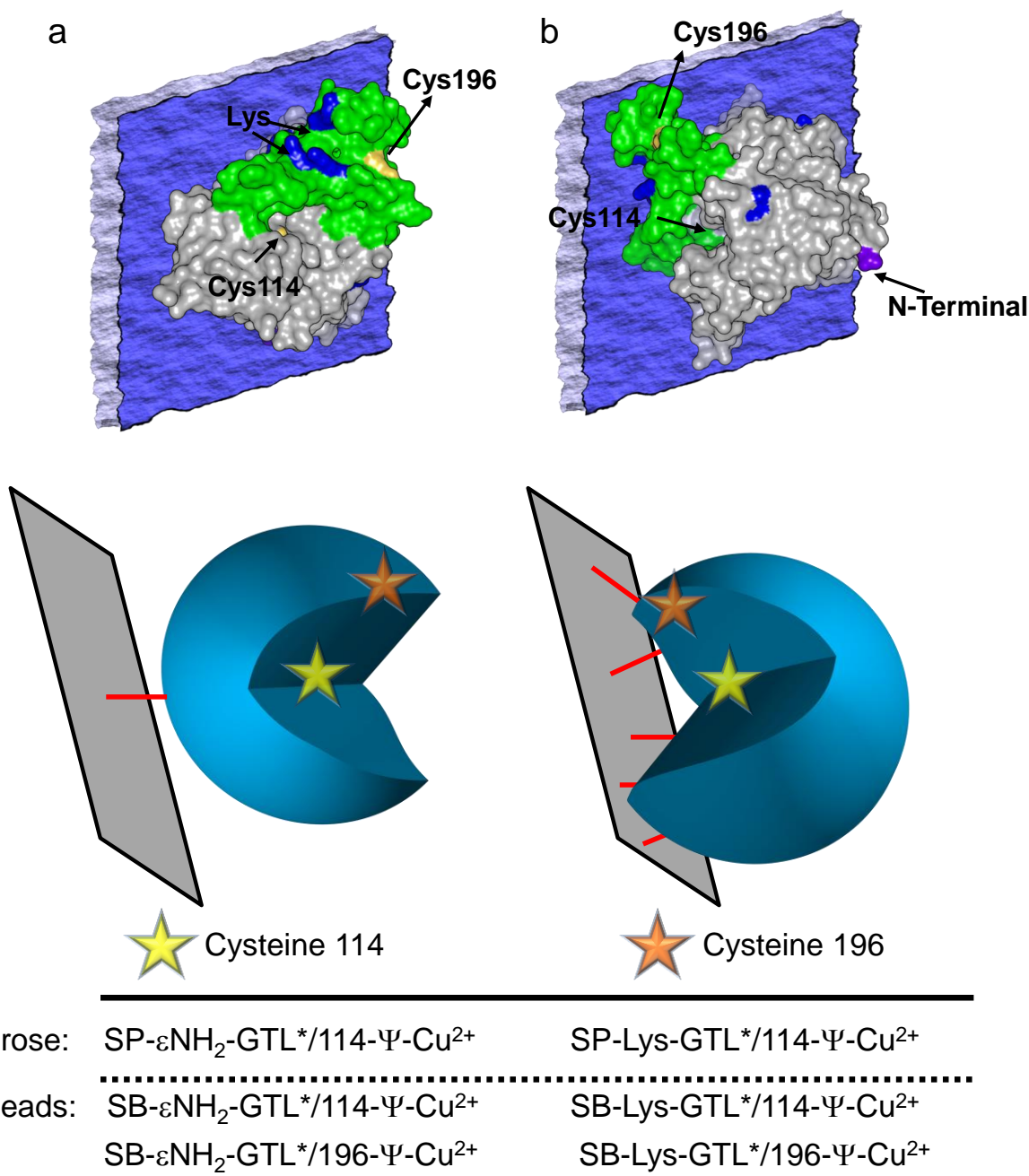| Sepharose:	SP-εNH$_2$-GTL*/114-Ψ-Cu^{2+}	SP-Lys-GTL*/114-Ψ-Cu^{2+}
Sepabeads:	SB-εNH$_2$-GTL*/114-Ψ-Cu^{2+}	SB-Lys-GTL*/114-Ψ-Cu^{2+}
	SB-εNH$_2$-GTL*/196-Ψ-Cu^{2+}	SB-Lys-GTL*/196-Ψ-Cu^{2+}

Figure S5. Different oriented immobilizations of artificial metallolipases on heterogeneous supports (Enzyme structure (PDB: 2W22)). a) Covalent immobilization through the terminal amino group; b) Multipoint covalent immobilization through the Lys-richest region.
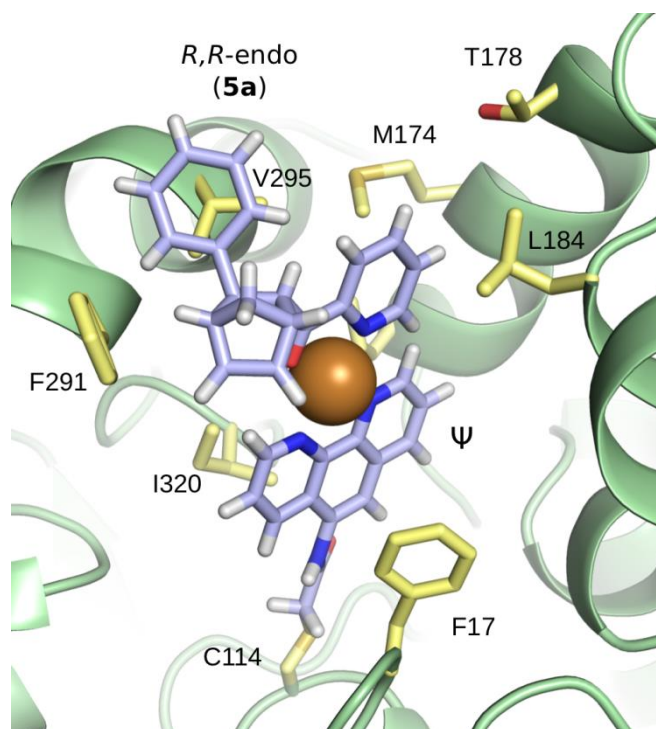
Figure S6. Stabilization of Ψ-Cu^{2+} and product **5a** in site 114 of GTL as obtained by docking calculations. The Ψ-Cu^{2+} and **5a** are represented in sticks (C atoms colored in light blue, O atoms in red, N atoms in dark blue and H in white). Cu^{2+} atom is represented as an orange sphere. The side-chains of residues interacting with ligands are represented as yellow sticks. The phenanthroline ring is mainly stabilized by Phe17 and Ile320 while other residues are involved in van der Waals interactions with ligand **5a**.
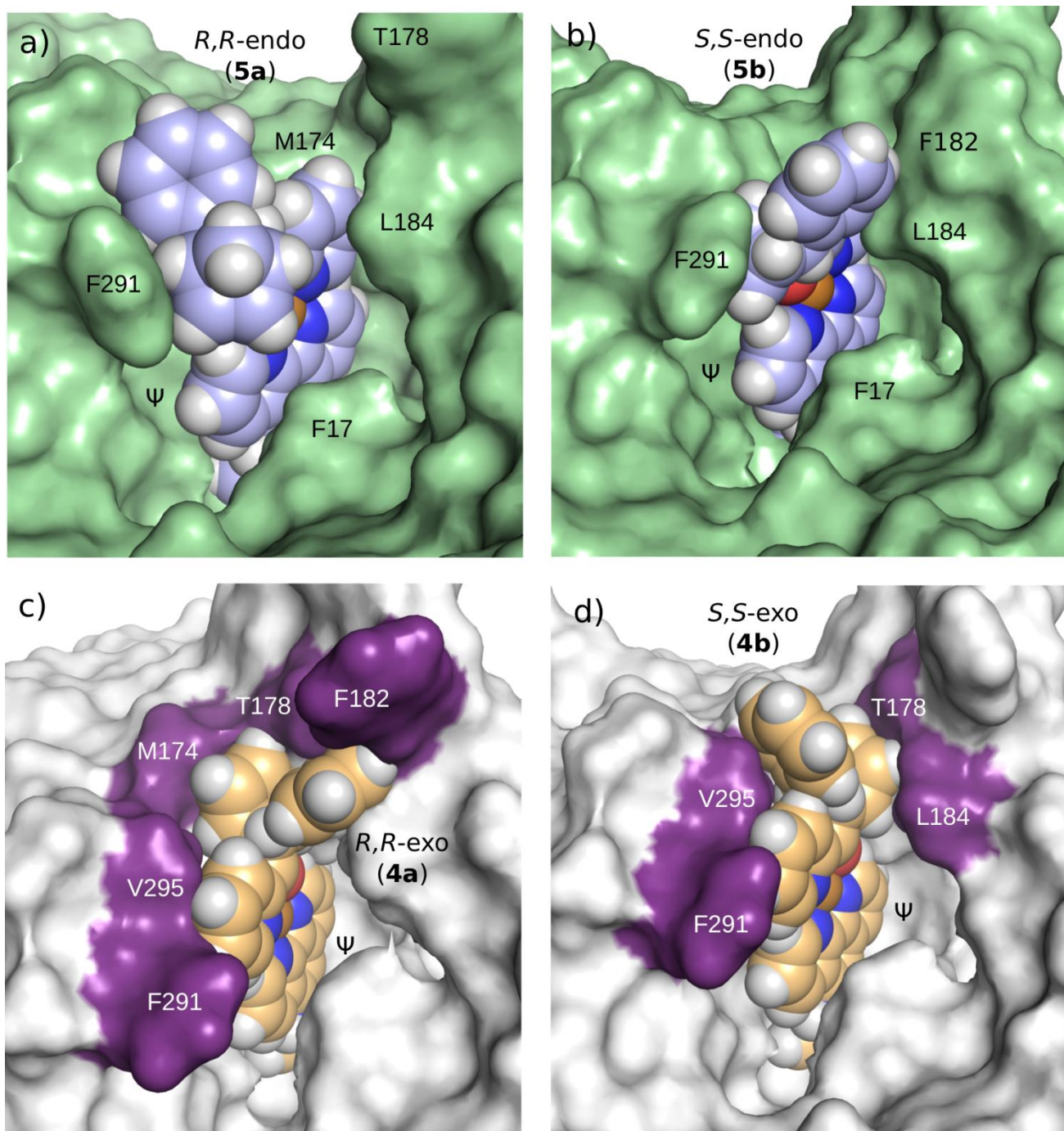
Figure S7. Docking results for **5a**, **5b**, **4a** and **4b** reaction products at site 114. a) Ligands Ψ-Cu^{2+} and **5a** are mainly stabilized by the interaction with aromatic residues Phe17 and Phe291 and with Leu184 and Met174. b) Ligands Ψ-Cu^{2+} and **5b** are mainly stabilized by the interaction with aromatic residues Phe17, Phe182, Leu184 and Phe291. In both **5a** and **5b** cases product release is favored by disposition of both ligands at site 114 and

flexibility of side chain from Phe291. c) Ligands Ψ-Cu^{2+} and **4a** at site 114 present many clashes with different residues (Met174, Thr178, Phe182, Phe291 and Val295) on GTL surface (colored in magenta). These residues would avoid the release of the product. d) Ligands Ψ-Cu^{2+} and **4b** at site 114 present many clashes with different residues (Phe291, Val295, Thr178 and Leu184 colored in magenta) on GTL surface. Disposition of ligand **4b** at site 114 suggests that reaction is not favored because the bicyclic ring is buried under Thr178 and Leu184.
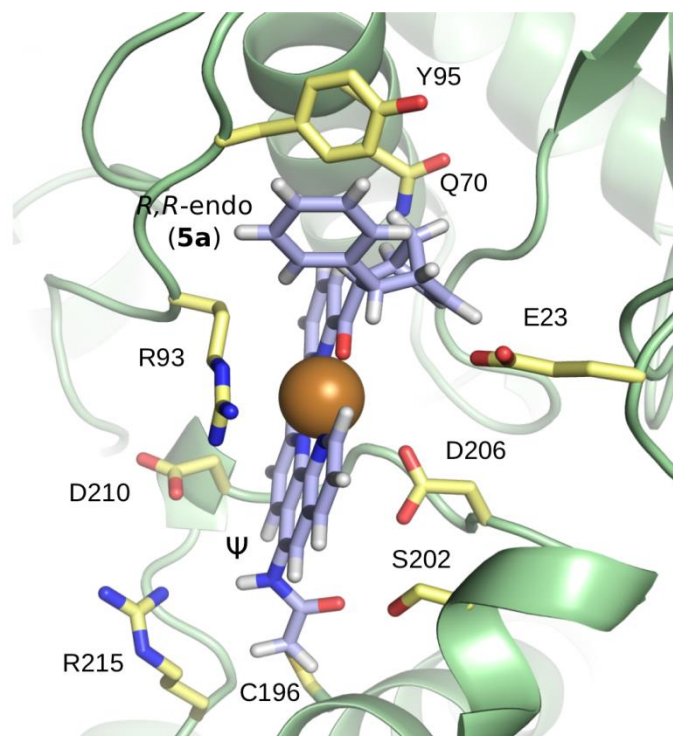
Figure S8. Docking result showing the attachment site and stabilization of Ψ-Cu^{2+} and product **5a** in site 196. The Ψ-Cu^{2+} and **5a** are represented in sticks (C atoms colored in light blue, O atoms in red, N atoms in dark blue and H in white). Cu^{2+} atom is represented as an orange sphere. The side-chain of residues interacting with ligands are represented in sticks (C atoms colored in yellow, O atoms in red and N atoms in dark blue) and labeled. The phenanthroline ring is stabilized by a cation/π interactions with Arg93. Other residues like Asp210 and Arg215 could also be involved in stabilization of Ψ-Cu^{2+}. Residues like Glu23, Gln70 and Tyr95 are involved in the stabilization of compound **5a** through polar interactions.
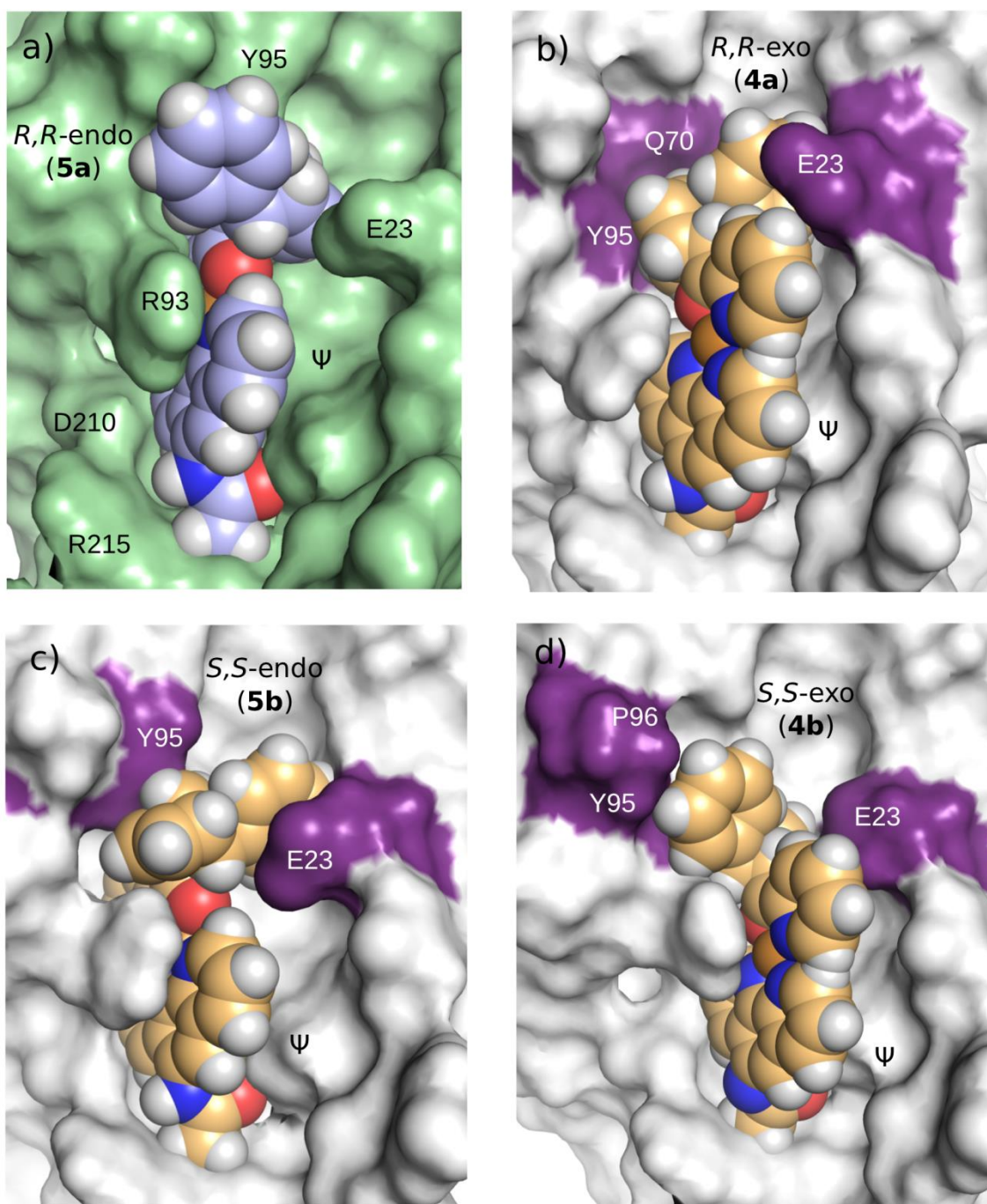
Figure S9. Docking results for **5a**, **5b**, **4a** and **4b** reaction products at site 196. a) Ligands Ψ-Cu^{2+} and **5a** are mainly stabilized by Glu23, Arg93 and Tyr95 on GTL surface. b) Ligands Ψ-Cu^{2+} and **4a** at site 196 present many clashes with different residues (Glu23, Gln70 and Tyr95) on GTL surface (colored in magenta). These residues would impede the release of the product. c) Ligands Ψ-Cu^{2+} and **5b** at site 196 present clashes with residues Gln23 and Tyr95 (colored in magenta). d) Ligands Ψ-Cu^{2+} and **4b** at site 196 present

clashes with residues Gln23, Tyr95 and Pro96 (colored in magenta). The bicyclic moiety of **4b** is buried and not accessible to the solvent, which could impede product formation.
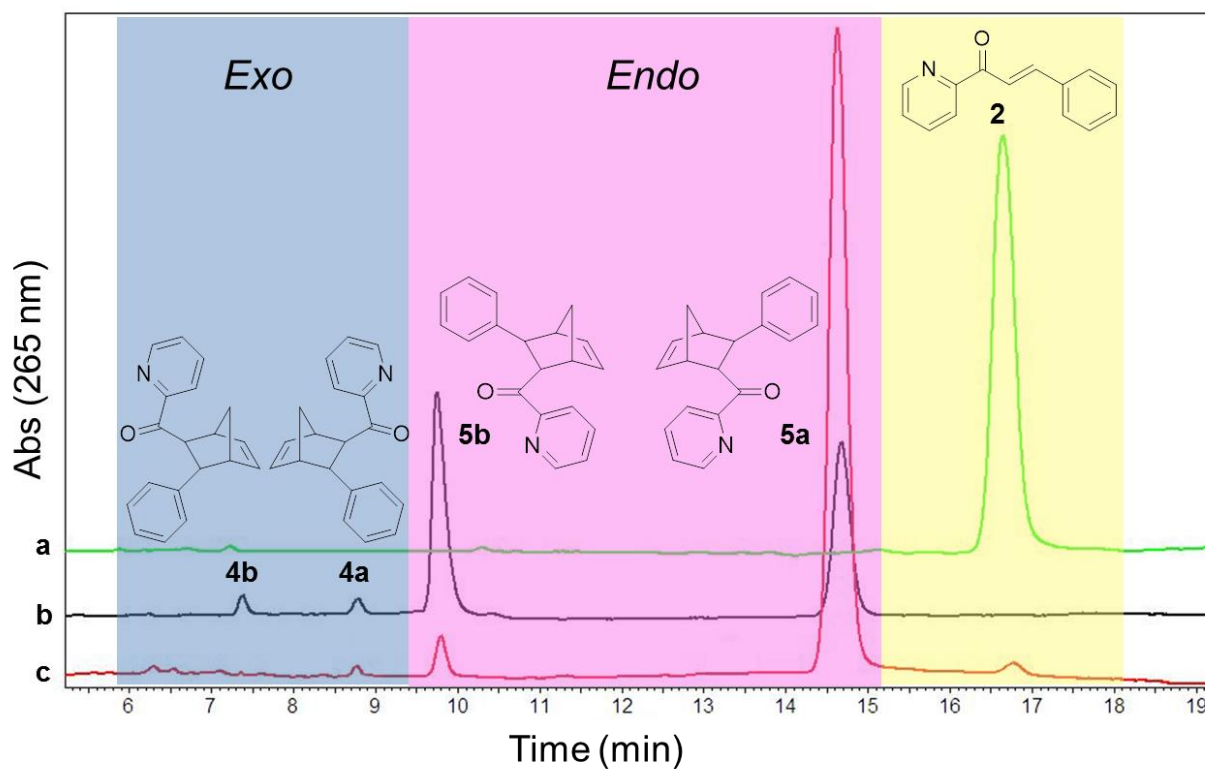
Figure S10. HPLC analysis of Diels-Alder cycloaddition reaction. a) HPLC trace of starting azachalcone **2** reactant. b) HPLC trace of standard Diels-Alder reaction catalyzed by soluble Cu^{2+} salt after *silica* purification of products. c) HPLC trace of Diels-Alder reaction catalyzed by SB-Lys-GTL*/196-Ψ-Cu^{2+} derivative.
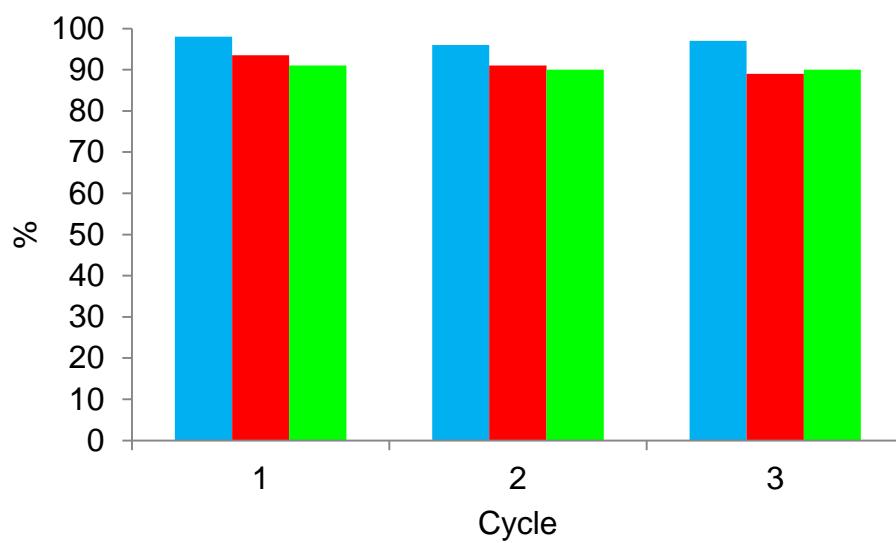
Figure S11. Recycling study of SB-Lys-GTL*/196-Ψ-Cu^{2+} heterogeneous artificial metallolipase. Bar color code: Blue: Conversion; Red: *endo/exo*; Green: *ee*.
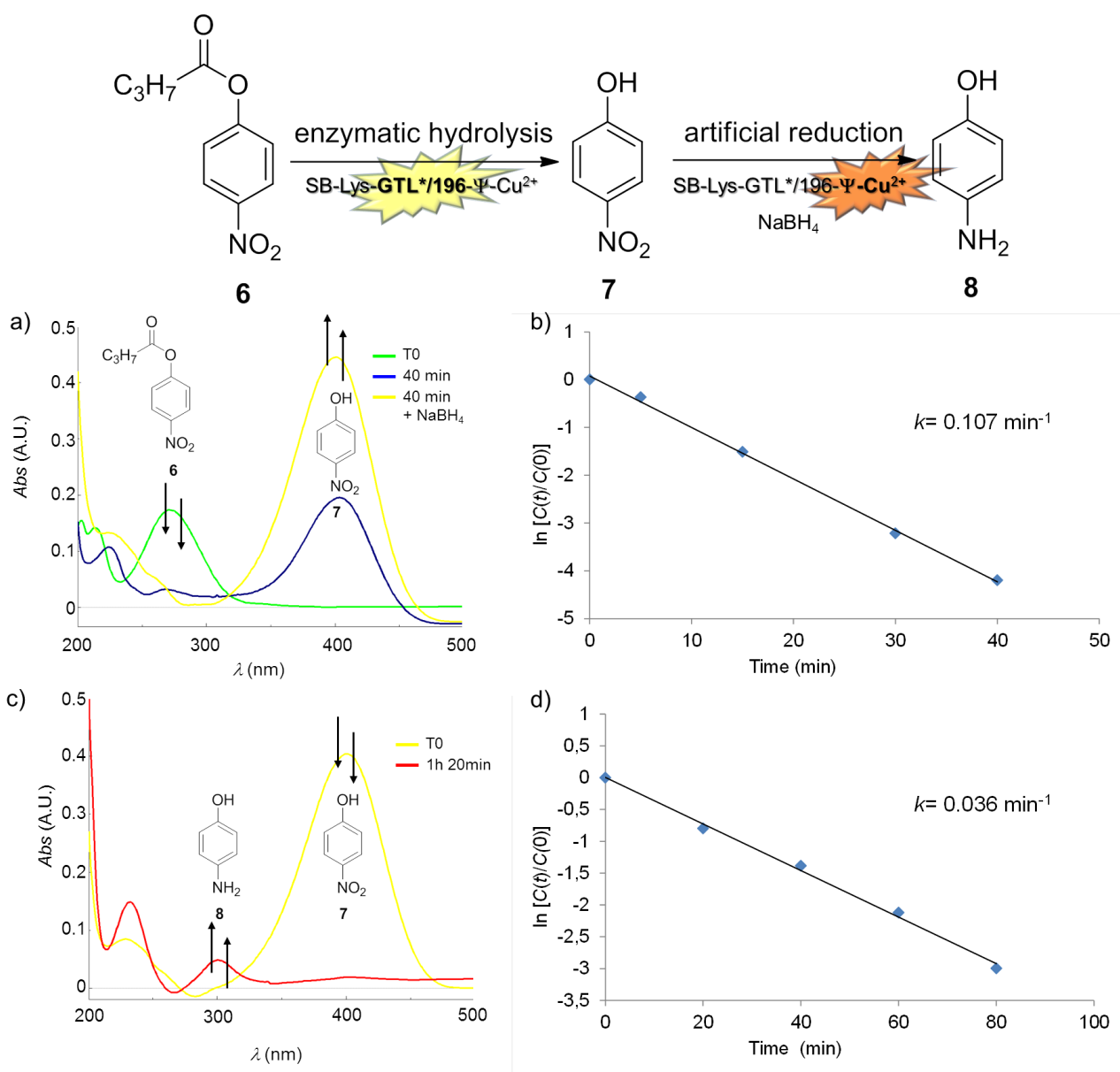
Figure S12. UV-Spectrophotometric evaluation of domino one-pot synthesis of aminoarene **8** starting from nitroarene ester **6** catalyzed by SB-Lys-GTL*/196-Ψ-Cu^{2+} artificial metallolipase. a) Time-dependent absorption spectra of the native enzyme-catalyzed hydrolysis of **6** in the presence of SB-Lys-GTL*/196-Ψ-Cu^{2+} artificial catalyst. b) Plot of ln[C(t)/C(0)] against the reaction time. The *k* value was calculated performing the linear relationship between lnC(t)/C(0) (with C(t) and C(0) representing the concentration

of product **6** at time t and 0, respectively) and reaction time. c) Time-dependent absorption

spectra of the Cu^{2+}-catalyzed reduction of **7** in the presence of SB-Lys-GTL*/196-Ψ-Cu^{2+}

artificial catalyst and $NaBH_4$. d) Plot of ln[C(t)/C(0)] against the reaction time. The *k* value

was calculated performing the linear relationship between lnC(t)/C(0) (with C(t) and C(0)

representing the concentration of product **7** at time t and 0, respectively) and reaction time.

Table S1. Preparation of different heterogeneous artificial metallolipase derivatives.

Entry	Hybrid catalysts	Immobilization Yield (%)	Protein loading $(mg_{prot}/g)^a$	Cu^{2+} loading ($\mu mol/g$)
1	SP-C4-GTL*/114-Ψ-Cu^{2+b}	97	10.7	244
2	SP-εNH_2-GTL*/114-Ψ-Cu^{2+}	85.5	9.4	223
3	SP-Lys-GTL*/114-Ψ-Cu^{2+}	92.7	10.2	242
4	SB-εNH_2-GTL*/114-Ψ-Cu^{2+}	89.1	9.8	233
5	SB-Lys-GTL*/114-Ψ-Cu^{2+}	94.5	10.4	247
8	SB-εNH_2-GTL*/196-Ψ-Cu^{2+}	84.2	9.3	221
9	SB-Lys-GTL*/196-Ψ-Cu^{2+}	91.8	10.1	240

[a] The immobilization yields were calculated by Bradford assay; offered enzyme: 11 mg.

[b] The modification yield with ligand **1** was 96% calculated by Ellman's assay.

Table S2. Diels-Alder cycloaddition reaction catalyzed by different heterogeneous artificial metallolipase derivatives.[a]

Entry	Hybrid catalysts	Conversion (%)[b]	endo/exo (%)[c]	ee (%)[d]
1	Ψ-Cu^{2+}	28	92	<5
2	GTL*/114-(Cu^{2+})[e]	0	nd	nd
3	GTL*/114-Ψ-Cu^{2+}	53	94	5
4	GTL*/196-Ψ-Cu^{2+}	<5	nd	nd
5	SP-εNH$_2$-GTL*/114-Ψ-Cu^{2+}	0	nd	nd
6	SP-Lys-GTL*/114-Ψ-Cu^{2+}	0	nd	nd
7	SB-εNH$_2$-GTL*/114-Ψ-Cu^{2+}	51	94	<5
8	SB-Lys-GTL*/114-Ψ-Cu^{2+}	99	98.7	18
9	SB-εNH$_2$-GTL*/196-Ψ-Cu^{2+}	<5	nd	nd
10	SB-Lys-GTL*/196-Ψ-Cu^{2+}	98	93.5	92

[a] All reactions were performed with 1 mM of azachalcone, 10 mM of freshly distilled cyclopentadiene, 100 mg of heterogeneous artificial biocatalyst (0.238% mol) at 4°C for 72h in 1ml of 5% ACN in 10 mM MES buffered solution pH6. [b] Conversion. The yields were calculated by HPLC. [c] Determined by HPLC. [d] Determined by HPLC. [e] Unmodified GTL*/114 mutant incubated with Cu^{2+} salt as negative control.