Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary information

Design of a gold nanoparticles site in an engineered lipase: an artificial metalloenzyme with enantioselective reductase-like activity†

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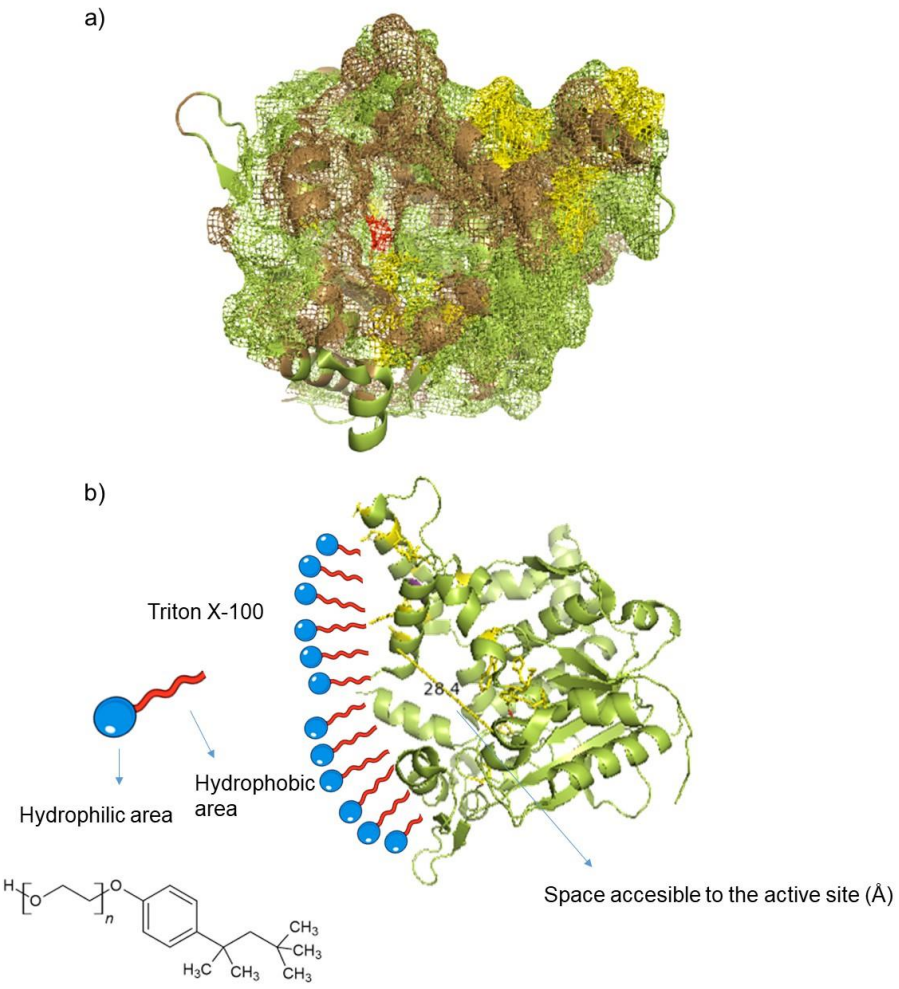

Fig. S1. A) Crystal structure cartoon of the open conformation of GTL, marked in yellow lid site and in brown hydrophobic residues. B) Representation of the stabilization of open conformation of GTL by triton X-100 molecules v*ia* hydrophobic interactions. The protein structure was obtained from the Protein Data Bank (pdb code: 2W22) and the picture was created using Pymol.

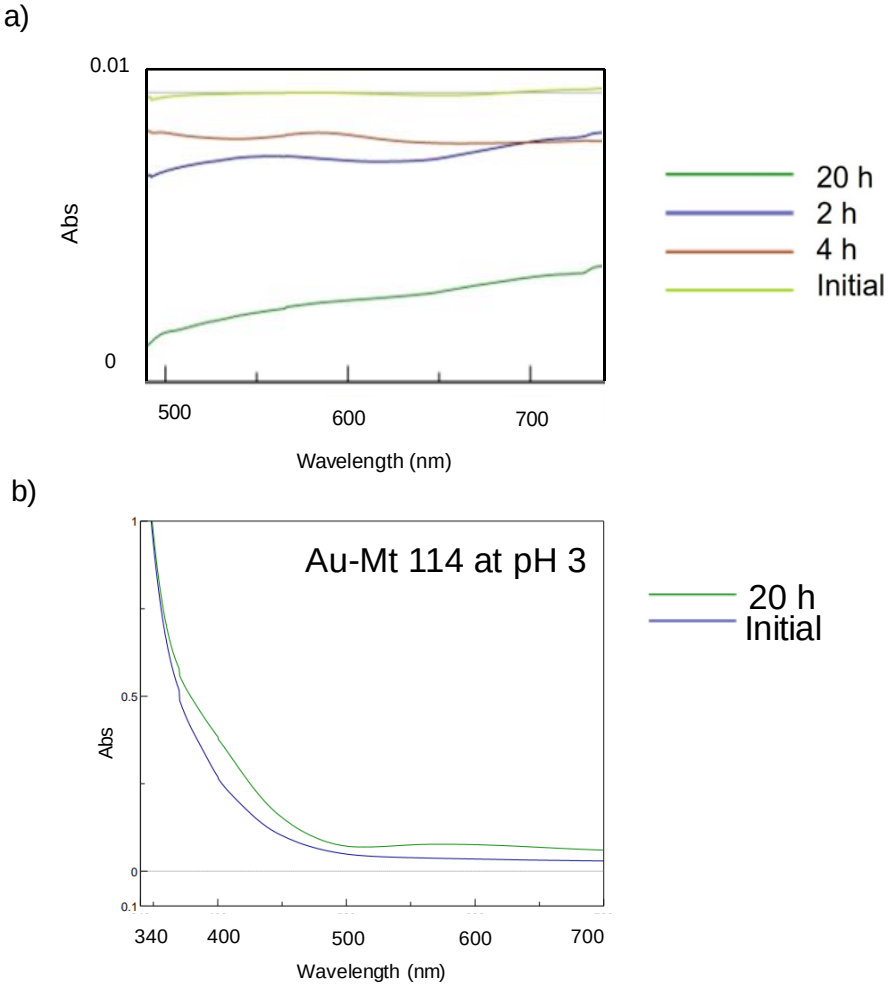

Fig. S2. UV-characterization of Enzyme-Au conjugates. a) pH 7 to 10 at r. t. b) pH 3 at r.t or 50ºC.

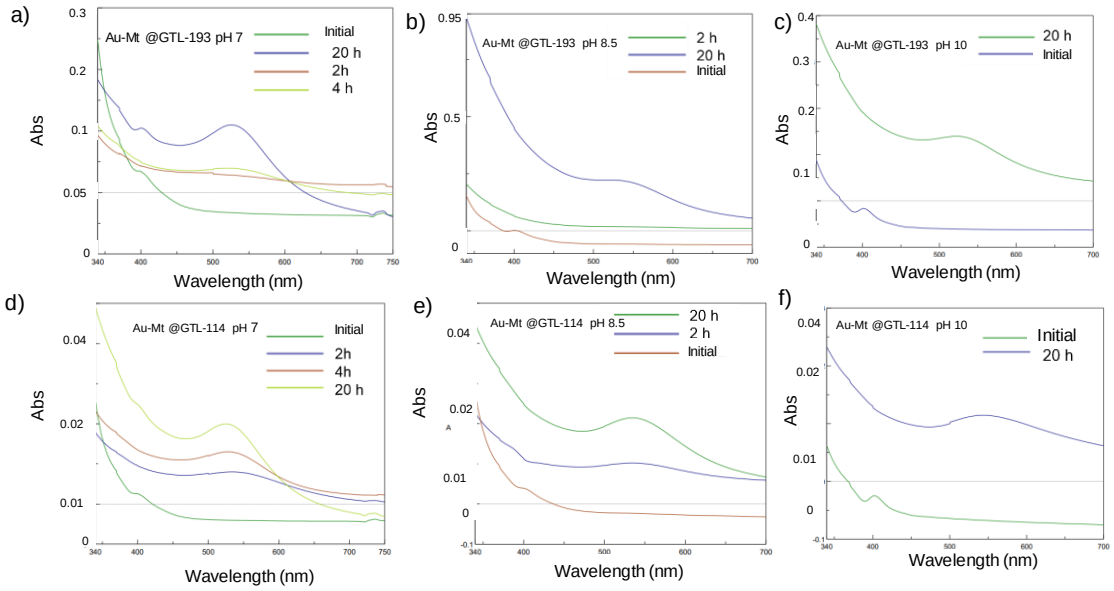

Fig. S3. UV-spectra profile in the synthesis of the gold metalloenzymes. **a) Au-Mt@GTL-193 pH 7. b) Au-Mt@GTL-193 pH 8.5. c) Au-Mt@GTL-193 pH 10. d) Au-Mt@GTL-114 pH 7. e) Au-Mt@GTL-114 pH 8.5. f) Au-Mt@GTL-114 pH 10.**

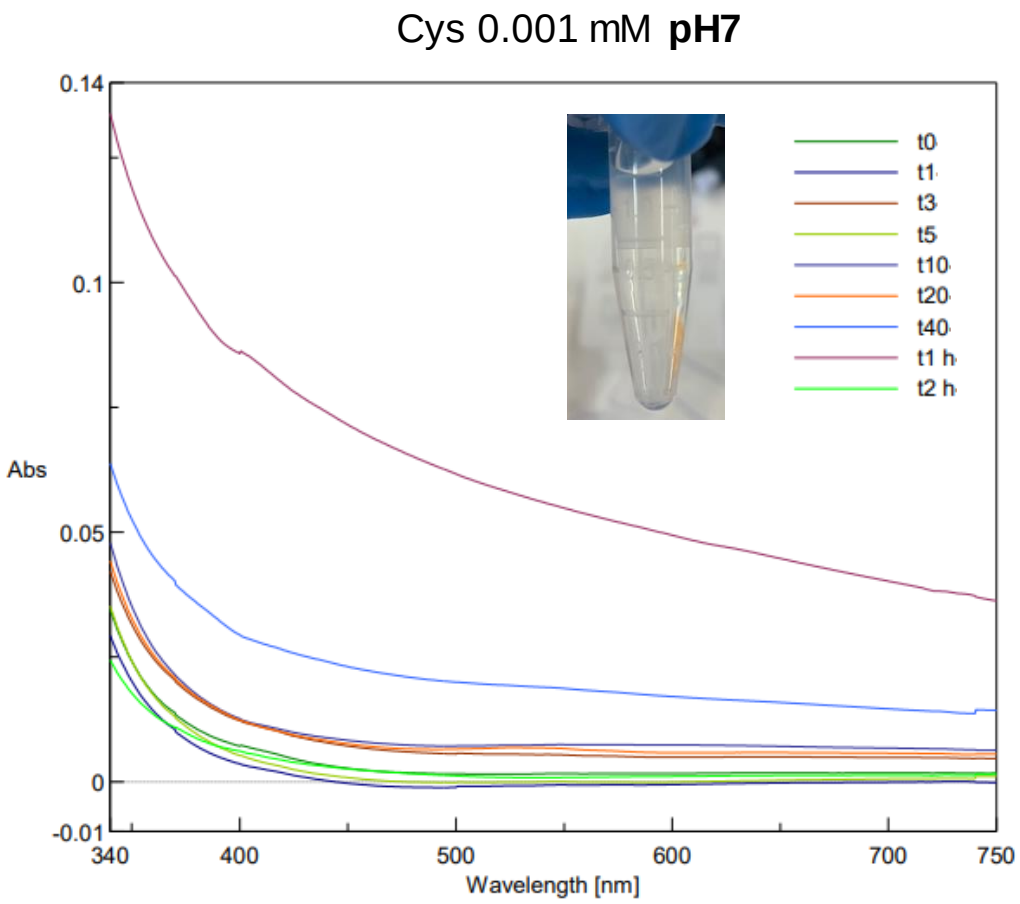

Fig S4. Test of AuNPs formation using L-Cys in aqueous media with gold salt without reducing agent (e.g. ascorbic acid).

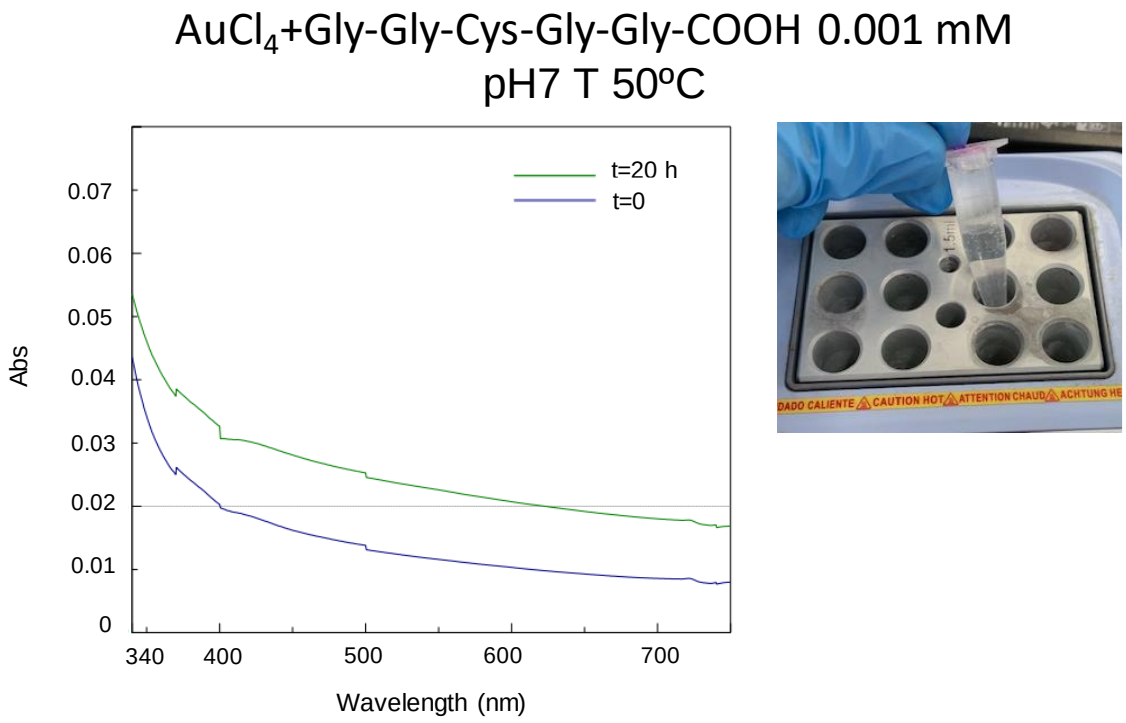

Fig S5. Test of AuNPs formation using Gly-Gly-Cys-Gly-Gly-Gly peptide in aqueous media with gold salt without reducing agent (e.g. ascorbic acid).

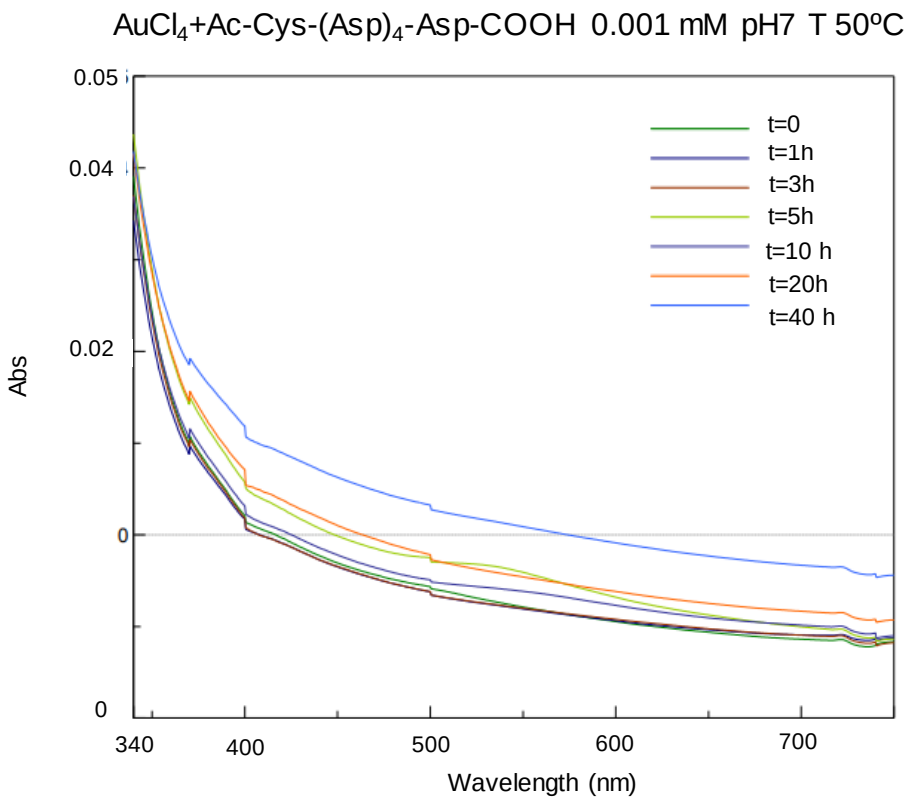

Fig S6. Test of AuNPs formation using Ac-Cys-(Asp)$_4$-Asp-COOH peptide in aqueous media with gold salt without reducing agent (e.g. ascorbic acid).

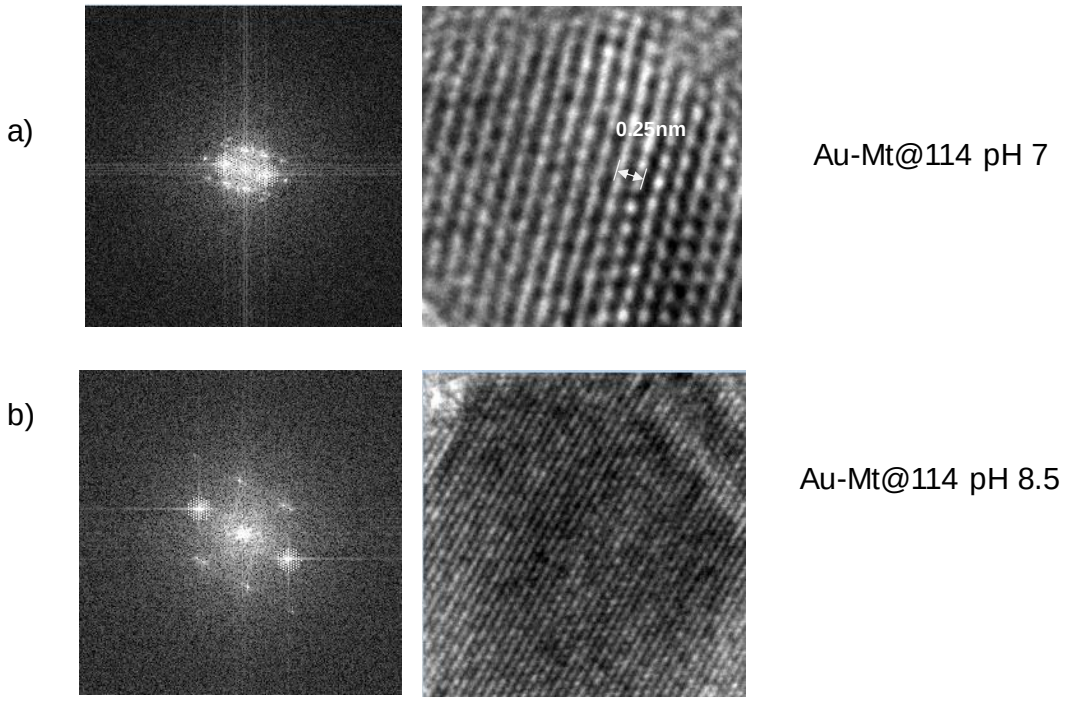

Fig S7. Fast Fourier transform (FFT) patterns from HRTEM images of GTL114 Au metalloenzymes.

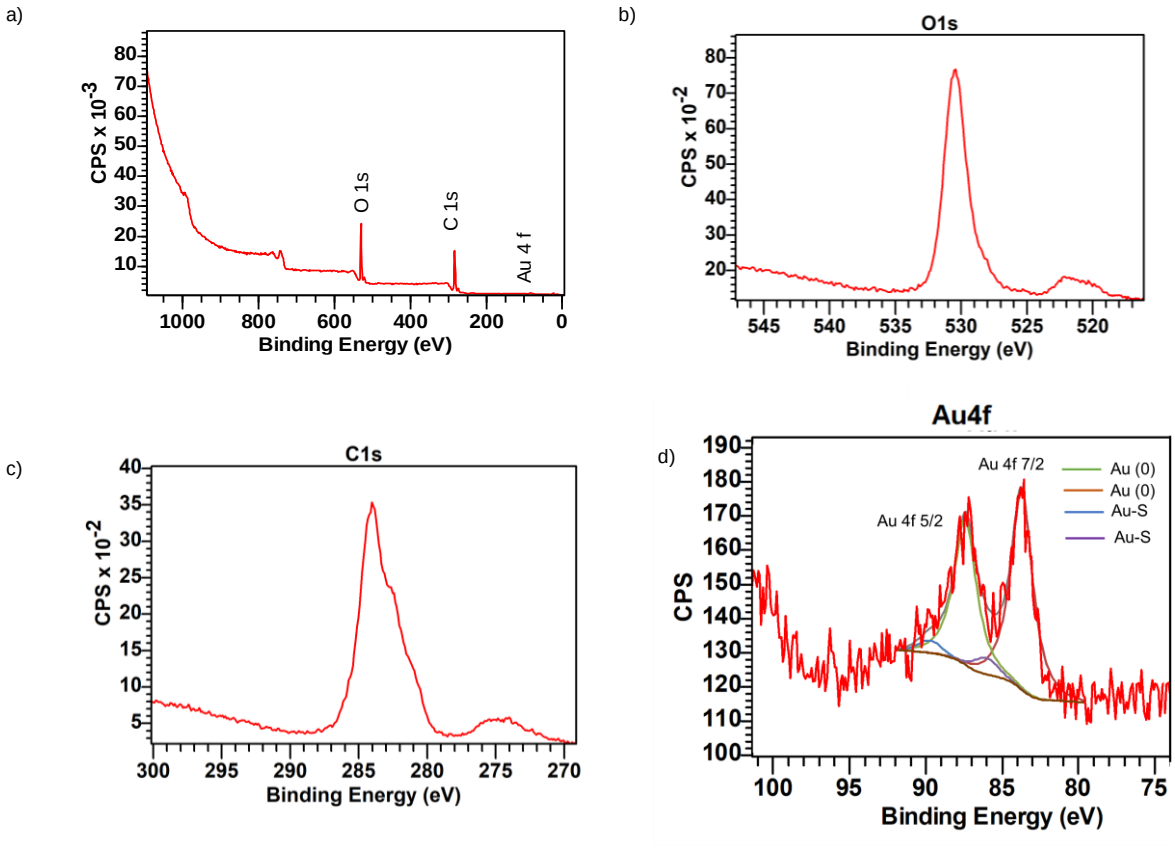

Fig S8. Characterization of **Au@GTL-193** by X-ray photoelectron spectroscopy (XPS). a) Full XPS spectrum; b) XPS spectrum of O1s; c) XPS spectrum of C1s; d) XPS spectrum of Au 4f. The fitted curve shows that the spectra comprise two doublets of Au(0) (green and orange) and Au-S(blue and purple).

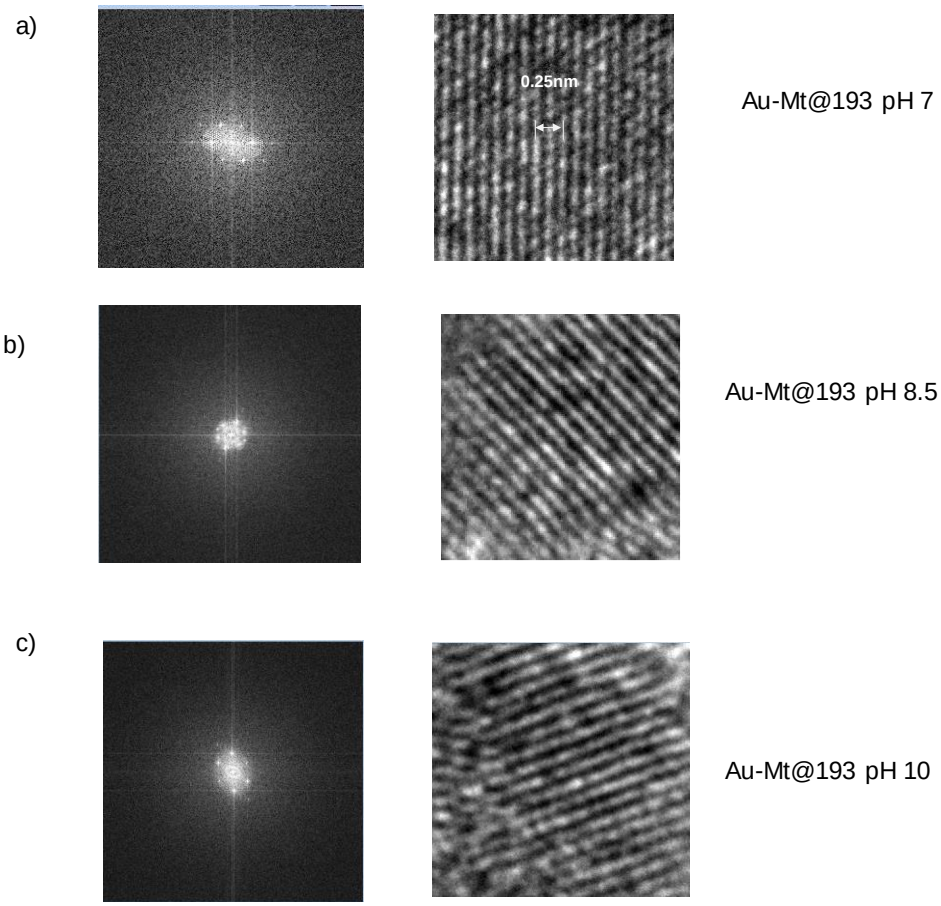

Fig S9. Fast Fourier transform (FFT) patterns from HRTEM images of GTL193 Au metalloenzymes.

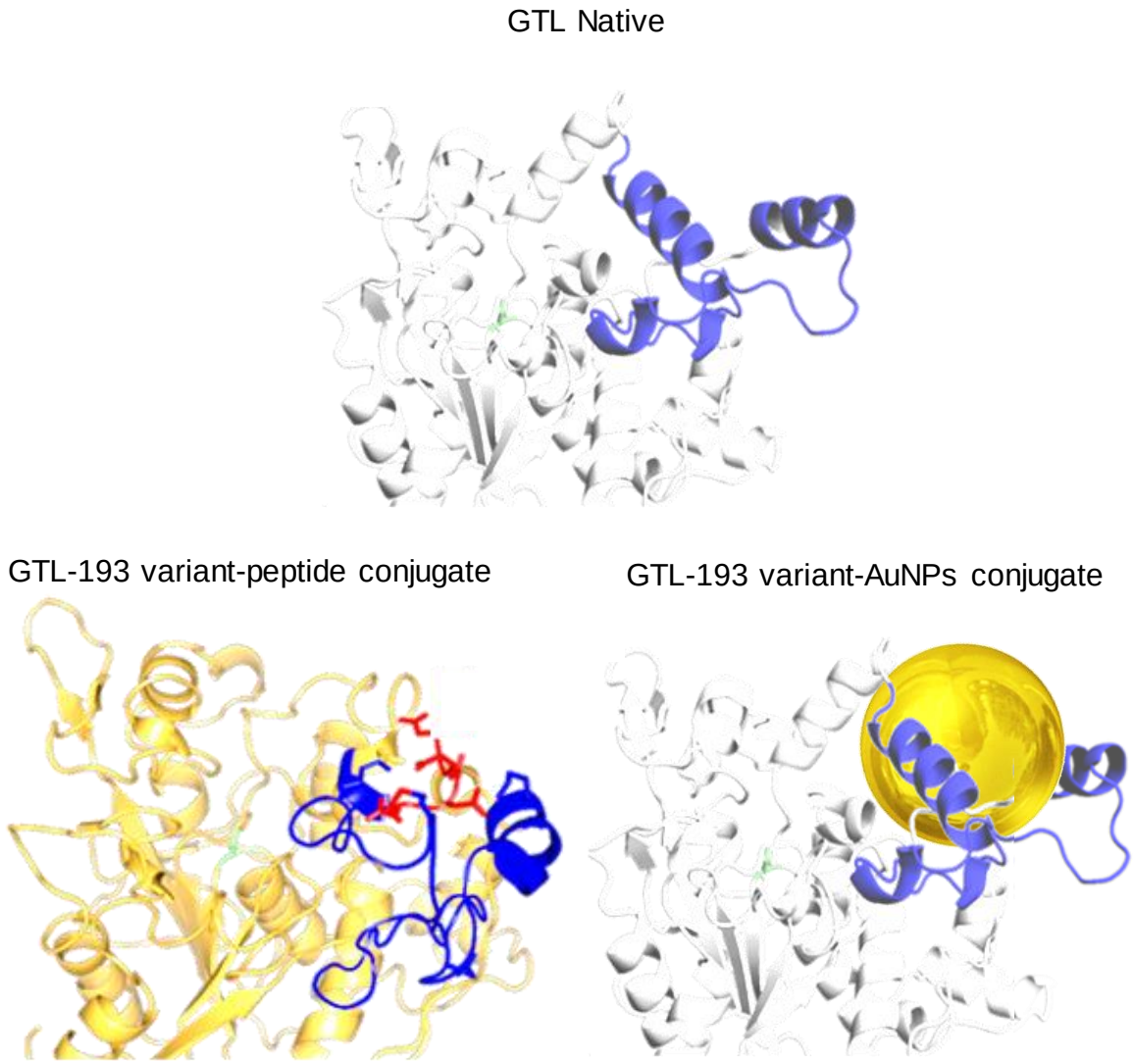

Fig S10. Comparison between crystal structure of the open conformation of GTL and the artificial enzymes GTL-193-peptide, previously created by using targeted Molecular Dynamic technique as implemented in AMBER 11 suite of programs and GTL-193-AuNPs. Corresponding structures taken from the PDB (PDB IDs 1JI3 and 2W22)[29] were used All pictures were created by using Pymol.

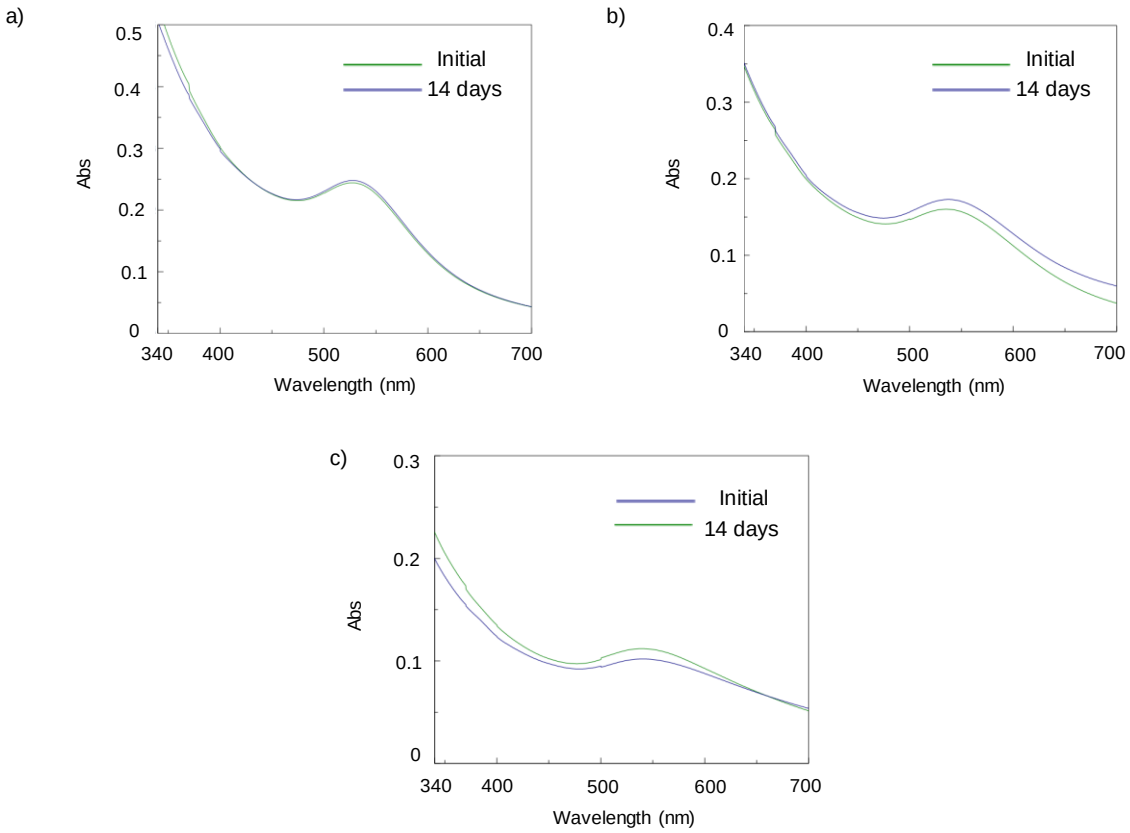

Fig S11. Stability studies of Au-Mt@114 metalloenzymes at 4ºC. UV spectra of a) Au-Mt@GTL-114 pH 7; b) Au-Mt@GTL-114 pH 8.5 and c) Au-Mt@GTL-114 pH 10.

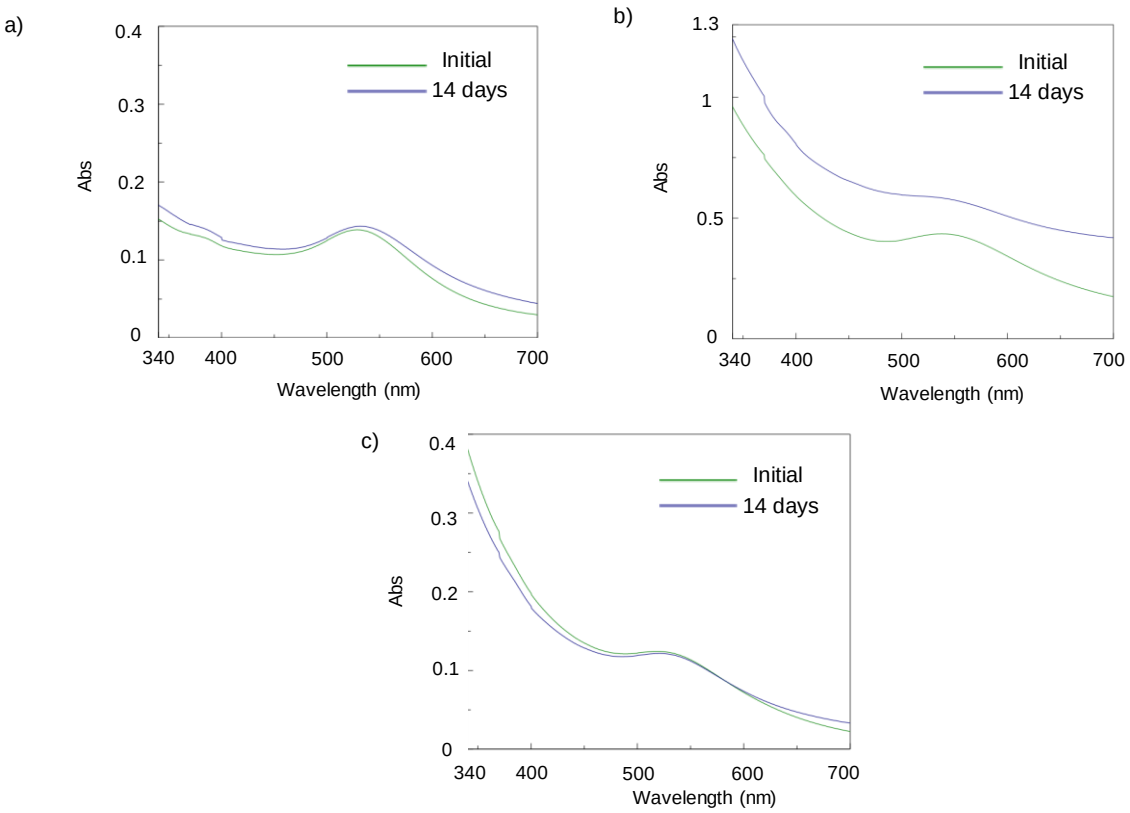

Fig S12. Stability studies of Au-Mt@193 metalloenzymes at 4ºC. UV spectra of a) Au-Mt@GTL-193 pH 7; b) Au-Mt@GTL-193 pH 8.5 and c) Au-Mt@GTL-193 pH 10.

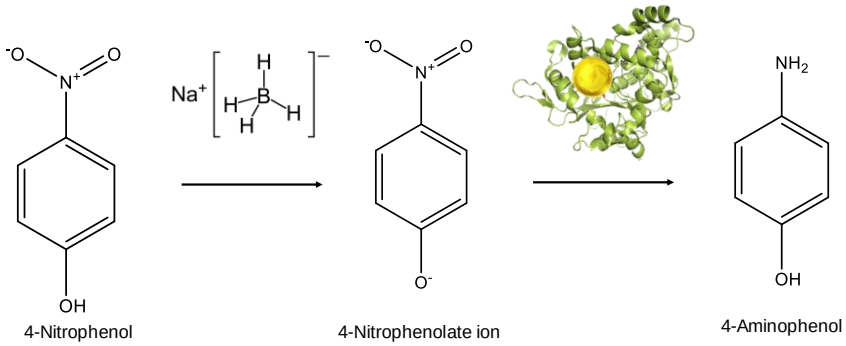

Fig S13. Proposed reaction mechanism of the reduction of p-nitrophenol catalysed by Au-metalloenzymes.

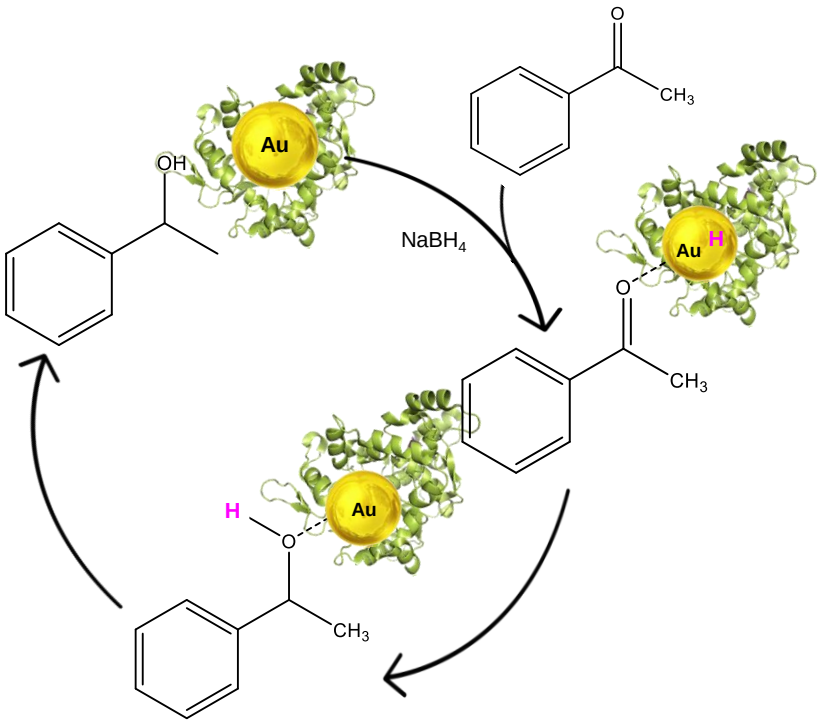

Fig S14. Proposed reaction mechanism of the asymmetric reduction of acetophenone catalysed by Au-metalloenzymes.

Table S1. Fluorescence and hydrolytic activity characterization of the different GTL variants.

Enzyme variant	λ emission (nm)	Specific activity[b]
Native GTL	303	2.76
C65S/C296S-GTL	304	2.76
GTL-C114	304	-[c]
GTL-C193	304	2.81

[a] Fluorescence maximum signal at conditions described in experimental. [b]0.4mM p-nitrophenylbutyrate in Phosphate Buffer 25mM pH 7.0 at 25ºC UI/$mg_{prot.}$. [c] enzyme variant is not active because mutation was performed in the serine (S114) active site.

Table S2. Fluorescence data for the gold metalloenzymes.

Entry	Sample	λ emission (nm)
1	**Free GTL-114/193**	303
2	**Au-Mt@GTL-114 pH 7**	310
3	**Au-Mt@GTL-114 pH 8.5**	305
4	**Au-Mt@GTL-114 pH 10**	307
5	**Au-Mt@GTL-193 pH 7**	307
7	**Au-Mt@GTL-193 pH 8.5**	307
8	**Au-Mt@GTL-193 pH 10**	310

Table S3. Protein and Au content of the gold metalloenzymes.

Entry	Sample	Protein concentration (mg/mL)	Au content (mg/mL)	Protein (nmol)	Au (nmol)
1	**Au-Mt@GTL-114 pH 7**	0.07	$8.3 \cdot 10^{-5}$	0.16	1.82
2	**Au-Mt@GTL-114 pH 8.5**	0.07	$6.4 \cdot 10^{-5}$	0.16	1.92
3	**Au-Mt@GTL-114 pH 10**	0.07	$6.3 \cdot 10^{-5}$	0.16	1.83
4	**Au-Mt@GTL-193 pH 7**	0.07	$7.1 \cdot 10^{-5}$	0.16	2.11
5	**Au-Mt@GTL-193 pH 8.5**	0.07	$7.5 \cdot 10^{-5}$	0.16	1.64
6	**Au-Mt@GTL-193 pH 10**	0.07	$7.2 \cdot 10^{-5}$	0.16	1.83

Table S4. Stability of gold metalloenzymes at 4ºC.

Entry	Sample	Initial		14 days	
		λ (nm)	**Abs**	**λ (nm)**	**Abs**
1	**Au-Mt@GTL-114 pH 7**	533	0.23	535	0.24
2	**Au-Mt@GTL-114 pH 8.5**	542	0.12	540	0.17
3	**Au-Mt@GTL-114 pH 10**	542	0.10	541	0.11
4	**Au-Mt@GTL-193 pH 7**	529	0.13	530	0.14
5	**Au-Mt@GTL-193 pH 8.5**	540	0.18	544	0.26
6	**Au-Mt@GTL-193 pH 10**	542	0.12	542	0.12