Supporting Information

Metal-binding sites of proteins drive the formation and affect the functional properties of bio-inorganic nanflowers of lipases.

Table S1. Predicted normalized scores applying the Frgament Transformation Method to the closed conformation of the lipase from *Thermomyces launiginosus* (TLL). Values highlighted in blue are the scores that meet the following criteria; residues with score value higher than 2.2 except the cysteine residues involved in disfulfide bridges and those residues fully buried (exposed area = 0 Å ^{2}). The residues highlighted in red are those ones that having scores higher than 2.2 are discarded due to the reason above described. The reasons to discard them (either being a cysteine or the exposed area) are also red colored in the table. The exposed area of each residue was calculated with Bluues server[1].

Position	Residue	Exposed area (Å^2)	Score Ca^{2+}	Score Cu^{2+}	Score Zn^{2+}
1	GLU	229.1	-0.321	-0.532	-0.343
2	VAL	23.22	-0.229	-0.532	-0.343
3	SER	122.5	-0.473	-0.532	-0.343
4	GLN	186.4	**2.926**	-0.532	-0.343
5	ASP	193.2	**2.926**	0.497	-0.343
6	LEU	61.7	-0.516	-0.532	-0.343
7	PHE	53.37	-0.473	-0.532	-0.343
8	ASN	161.5	0.558	0.497	-0.343
9	GLN	60.24	-0.19	-0.532	-0.343
10	PHE	0	-0.599	-0.532	-0.343
11	ASN	45.62	-0.239	-0.532	-0.343
12	LEU	35.77	-0.599	-0.532	-0.343
13	PHE	2.888	-0.97	0.799	-0.343
14	ALA	0	-0.046	-0.532	-0.343
15	GLN	25.89	0.075	-0.532	-0.343
16	TYR	0	-0.046	0.958	-0.343
17	SER	0	-0.443	-0.532	-0.343
18	ALA	4.868	-1.629	-0.532	-0.343
19	ALA	0	0.508	-0.532	-0.343
20	ALA	0	0.52	-0.532	-0.343
21	TYR	35.43	0.508	0.899	-0.343
22	**CYS**	**0**	-0.644	-0.532	**3.226**
23	GLY	41.33	0.508	-0.532	-0.343
24	LYS	212.2	0.2	-0.532	-0.343
25	ASN	0	**1.943**	-0.532	-0.343
26	ASN	85.96	**1.943**	1.579	-0.343
27	ASP	200.5	0.927	1.579	-0.343
28	ALA	35.45	0.404	-0.532	-0.343
29	PRO	200.5	-0.723	-0.532	-0.343
30	ALA	153.1	0.235	-0.532	-0.343

31	GLY	103.9	0.235	-0.532	-0.343
32	THR	93.63	-1.374	-0.532	-0.343
33	ASN	190.8	0.235	-0.532	-0.343
34	ILE	0	-0.022	-0.532	-0.343
35	THR	84.63	0.508	**2.518**	-0.343
36	**CYS**	26.57	-0.718	**2.518**	**3.226**
37	THR	118	-0.945	1.571	-0.343
38	GLY	88.87	-1.132	-0.532	-0.343
39	ASN	248.1	-0.718	-0.532	-0.343
40	ALA	15.5	-0.441	-0.532	-0.343
41	**CYS**	**0**	-0.903	**2.518**	**2.278**
42	PRO	77.32	0.626	-0.532	-0.343
43	GLU	104.9	0.885	-0.532	-0.343
44	VAL	0	0.626	-0.532	-0.343
45	GLU	129.3	0.626	-0.532	-0.343
46	LYS	316.4	0.222	-0.532	-0.343
47	ALA	12.57	0.599	-0.532	-0.343
48	ASP	153.4	0.885	-0.532	-0.343
49	ALA	0	0.885	-0.532	-0.343
50	THR	29.14	0.52	-0.532	-0.343
51	PHE	0	-0.272	-0.532	-0.343
52	LEU	97.23	-0.724	-0.532	-0.343
53	TYR	119.1	0.981	-0.532	-0.343
54	SER	17.32	-0.339	-0.532	-0.343
55	PHE	7.104	0.52	-0.532	-0.343
56	GLU	136.9	1.943	1.222	1.936
57	ASP	135	0.521	1.222	2.11
58	SER	43.26	0.232	-0.532	**2.968**
59	GLY	66.87	0.521	-0.532	-0.343
60	VAL	238.1	-0.502	-0.532	-0.343
61	GLY	70.23	0.416	2.18	-0.343
62	ASP	152.1	0.416	2.18	**2.968**
63	VAL	5.245	-0.148	-0.532	-0.343
64	THR	0	0.168	-0.532	-0.343
65	GLY	0	0.52	-0.532	-0.343
66	PHE	0	0.52	-0.532	-0.343
67	LEU	0	-1.13	-0.532	-0.343
68	ALA	0	-0.989	-0.532	-0.343
69	LEU	34.52	-0.272	-0.532	-0.343
70	ASP	**0**	0.89	-0.532	**3.387**
71	ASN	97.03	0.885	0.909	-0.343
72	THR	186	0.89	-0.532	-0.343
73	ASN	134.7	0.599	0.833	-0.343

74	LYS	213	0.885	0.833	-0.343
75	LEU	9.297	-0.114	-0.532	-0.343
76	ILE	0	-0.989	-0.532	-0.343
77	VAL	0	-1.401	-0.532	-0.343
78	LEU	0	-3.205	-0.532	-0.343
79	SER	0	-0.524	-0.532	-0.343
80	PHE	0	-0.948	-0.532	-0.343
81	ARG	23.65	0.508	-0.532	-0.343
82	GLY	0	-0.842	-0.532	-0.343
83	SER	32.55	0.46	-0.532	-0.343
84	ARG	321	-0.62	-0.532	-0.343
85	SER	140.3	0.46	-0.532	-0.343
86	ILE	13.05	-1.23	-0.532	-0.343
87	GLU	244	0.225	-0.532	-0.343
88	ASN	191.1	0.268	-0.532	-0.343
89	TRP	64.58	-0.906	-0.532	-0.343
90	ILE	36.18	-0.975	-0.532	-0.343
91	GLY	152.8	1.047	-0.532	-0.343
92	ASN	238.9	0.268	-0.532	-0.343
93	LEU	40.96	1.047	-0.532	-0.343
94	ASN	161.4	1.249	-0.532	-0.343
95	PHE	94.48	1.249	-0.532	-0.343
96	ASP	190.2	**2.297**	-0.532	1.989
97	LEU	69.74	**2.297**	-0.532	1.915
98	LYS	124.5	1.077	-0.532	1.989
99	GLU	185.1	0.931	-0.532	-0.343
100	ILE	0	0.761	-0.532	-0.343
101	ASN	163.9	**2.802**	**2.457**	-0.343
102	ASP	248.6	**2.802**	**2.457**	2.059
103	ILE	34.78	0.287	**2.964**	-0.343
104	**CYS**	34.49	-1.619	**2.964**	**2.327**
105	SER	203.5	-1.335	-0.532	-0.343
106	GLY	101.4	0.761	**2.426**	-0.343
107	**CYS**	2.854	-0.901	**2.964**	**2.327**
108	ARG	205.8	1.773	-0.532	-0.343
109	GLY	0	1.773	**3.169**	-0.343
110	HIS	8.445	-0.201	**3.169**	1.907
111	ASP	91.32	0.681	1.522	1.907
112	GLY	58.59	-0.418	0.86	-0.343
113	PHE	5.702	-0.198	-0.532	-0.343
114	THR	0	0.019	-0.532	-0.343
115	SER	66.45	0.209	-0.532	-0.343
116	SER	12.63	-0.752	-0.532	-0.343

117	TRP	0	-0.402	-0.532	-0.343
118	ARG	177.7	0.159	-0.532	-0.343
119	SER	100.8	0.159	-0.532	-0.343
120	VAL	0	-0.738	-0.532	-0.343
121	ALA	0	-0.916	-0.532	-0.343
122	ASP	228.5	0.159	-0.532	-0.343
123	THR	112.2	-0.122	-0.532	-0.343
124	LEU	0	-0.738	-0.532	-0.343
125	ARG	153	-0.217	-0.532	-0.343
126	GLN	147.8	0.386	-0.532	-0.343
127	LYS	102.8	0.552	-0.532	-0.343
128	VAL	0	-0.387	-0.532	-0.343
129	GLU	128.1	1.857	-0.532	-0.343
130	ASP	110.7	1.857	-0.532	**4.549**
131	ALA	3.385	-0.259	-0.532	-0.343
132	VAL	51.69	0.142	-0.532	-0.343
133	ARG	320	1.857	-0.532	-0.343
134	GLU	222.5	1.285	-0.532	**4.549**
135	HIS	72.08	-0.692	0.909	**3.387**
136	PRO	236.6	-0.412	1.574	-0.343
137	ASP	190.7	0.142	1.574	**3.387**
138	TYR	55.29	0.142	-0.532	-0.343
139	ARG	137.3	-0.689	-0.532	-0.343
140	VAL	4.456	0.479	-0.532	-0.343
141	VAL	0	-2.05	-0.532	-0.343
142	PHE	0	-0.84	-0.532	-0.343
143	THR	0	-0.345	-0.532	-0.343
144	GLY	0	-0.732	-0.532	-0.343
145	HIS	**0**	-0.443	2.006	**2.426**
146	SER	0	0.321	1.253	-0.343
147	LEU	21.77	-1.355	-0.532	-0.343
148	GLY	0	-0.842	-0.532	-0.343
149	GLY	0	-0.345	-0.532	-0.343
150	ALA	0	-1.488	-0.532	-0.343
151	LEU	0	-0.255	-0.532	-0.343
152	ALA	0	-1.355	-0.532	-0.343
153	THR	1.859	-1.054	-0.532	-0.343
154	VAL	0	0.287	-0.532	-0.343
155	ALA	0	-1.147	-0.532	-0.343
156	GLY	0	-0.89	-0.532	-0.343
157	ALA	7.246	-0.921	**2.577**	-0.343
158	ASP	56.52	0.287	**2.577**	2.059
159	LEU	0	0.16	-0.532	-0.343

160	ARG	26.57	-0.217	-0.532	-0.343
161	GLY	154.2	-0.289	-0.532	-0.343
162	ASN	136.2	0.16	-0.532	-0.343
163	GLY	176.8	0.65	**2.255**	-0.343
164	TYR	45.84	0.65	**2.255**	-0.343
165	ASP	184	1.951	-0.532	**3.074**
166	ILE	0	1.242	-0.532	-0.343
167	ASP	31.4	1.951	-0.532	**3.074**
168	VAL	0	0.675	-0.532	-0.343
169	PHE	0	-1.35	-0.532	-0.343
170	SER	0	-0.345	-0.532	-0.343
171	TYR	0	-0.97	1.33	-0.343
172	GLY	0	-1.024	-0.532	-0.343
173	ALA	1.859	-0.842	1.54	-0.343
174	PRO	2.415	-1.184	-0.532	-0.343
175	ARG	5.55	-1.292	-0.532	1.957
176	VAL	8.253	-0.825	0.712	-0.343
177	GLY	0	1.773	0.86	-0.343
178	ASN	45.84	1.773	-0.532	-0.343
179	ARG	253.2	-1.304	-0.532	-0.343
180	ALA	70.53	-0.576	-0.532	-0.343
181	PHE	0	-0.442	-0.532	-0.343
182	ALA	3.385	-0.386	-0.532	-0.343
183	GLU	128.8	-0.386	-0.532	-0.343
184	PHE	108.9	-1.264	1.391	-0.343
185	LEU	12.11	-1.806	-0.532	-0.343
186	THR	98.02	0.132	-0.532	-0.343
187	VAL	215	-1.04	-0.532	-0.343
188	GLN	31.12	-0.355	-0.532	-0.343
189	THR	256.1	0.81	-0.532	-0.343
190	GLY	41.92	-0.044	-0.532	-0.343
191	GLY	40.68	0.479	-0.532	-0.343
192	THR	144	0.675	-0.532	-0.343
193	LEU	16.42	0.675	-0.532	-0.343
194	TYR	55.05	-0.966	0.799	-0.343
195	ARG	11.96	-1.015	-0.532	-0.343
196	ILE	0	-3.205	-0.532	-0.343
197	THR	**0**	1.161	-0.532	**3.636**
198	HIS	**0**	1.161	1.8	**3.636**
199	THR	41.16	0.804	-0.532	-0.343
200	ASN	79.96	0.804	1.102	-0.343
201	ASP	0	0.793	-0.532	**2.426**
202	ILE	53.11	0.793	-0.532	-0.343

203	VAL	0	-0.609	1.54	-0.343
204	PRO	0	-0.622	-0.532	-0.343
205	ARG	47.95	-0.738	-0.532	-0.343
206	LEU	39.22	-1.199	-0.532	-0.343
207	PRO	0	-1.292	-0.532	-0.343
208	PRO	60.08	-0.539	-0.532	-0.343
209	ARG	192.4	0.368	0.451	-0.343
210	GLU	322.2	0.368	-0.532	1.957
211	PHE	235.1	-1.14	1.288	-0.343
212	GLY	77.24	-0.583	**2.616**	-0.343
213	TYR	**0**	-0.391	**2.616**	-0.343
214	SER	2.415	0.132	1.152	-0.343
215	HIS	10.2	0.235	1.288	-0.343
216	SER	3.307	0.289	-0.532	-0.343
217	SER	13.57	0.132	-0.532	-0.343
218	PRO	44.22	-1.548	-0.532	-0.343
219	GLU	0	0.289	-0.532	-0.343
220	TYR	19.58	-1.458	-0.532	-0.343
221	TRP	93.28	0.12	-0.532	-0.343
222	ILE	0	-1.17	-0.532	-0.343
223	LYS	194.5	0.361	-0.532	-0.343
224	SER	24.36	0.361	-0.532	-0.343
225	GLY	54.08	-1.093	-0.532	-0.343
226	THR	82.24	-0.276	-0.532	-0.343
227	LEU	263.7	-1.497	-0.532	-0.343
228	VAL	139.6	-1.143	-0.532	-0.343
229	PRO	218	-0.378	-0.532	-0.343
230	VAL	19.8	-0.557	-0.532	-0.343
231	THR	136.6	0.017	-0.532	-0.343
232	ARG	213.4	0.017	-0.532	-0.343
233	ASN	167.1	0.202	-0.532	-0.343
234	ASP	60.38	0.202	-0.532	-0.343
235	ILE	0	0.202	-0.532	-0.343
236	VAL	67.28	0.202	-0.532	-0.343
237	LYS	197.5	0.534	-0.532	-0.343
238	ILE	36.16	-0.176	-0.532	-0.343
239	GLU	253.5	0.534	-0.532	-0.343
240	GLY	68.74	0.853	-0.532	-0.343
241	ILE	69.59	0.853	-0.532	-0.343
242	ASP	31.28	0.853	-0.532	-0.343
243	ALA	38.4	0.098	-0.532	-0.343
244	THR	211.7	0.289	-0.532	-0.343
245	GLY	70.51	-0.206	-0.532	-0.343

246	GLY	0	0.057	1.404	-0.343
247	ASN	0	0.26	1.404	-0.343
248	ASN	38.16	0.26	-0.532	-0.343
249	GLN	100.3	0.314	-0.532	-0.343
250	PRO	273.8	-0.226	-0.532	-0.343
251	ASN	154.2	0.804	-0.532	-0.343
252	ILE	329.1	0.804	-0.532	-0.343
253	PRO	90.24	-0.622	0.782	-0.343
254	ASP	99.75	**2.283**	0.86	-0.343
255	ILE	93.2	**2.283**	-0.532	-0.343
256	PRO	135.3	1.317	-0.532	-0.343
257	ALA	0	0.453	1.998	-0.343
258	HIS	0	0.321	1.998	-0.343
259	LEU	81.8	1.317	0.701	-0.343
260	TRP	62.8	-1.093	1.8	-0.343
261	TYR	4.831	0.075	2.006	-0.343
262	PHE	42.4	0.075	1.8	-0.343
263	GLY	32.23	0.075	-0.532	-0.343
264	LEU	65.8	-1.132	-0.532	-0.343
265	ILE	0	-3.205	-0.532	-0.343
266	GLY	19.32	-0.842	0.987	-0.343
267	THR	100.6	-1.657	1.571	-0.343
268	CYS	114.2	-1.699	1.571	**3.226**
269	LEU	362.4	-0.945	1.34	-0.343

Table S2. Mineralisation Parameters of zinc-phosphate NFs using different Zn salts and different concentrations of metallic salts and sodium chloride.

Metallic Salt /(mM)	NaCl (mM)	Hybrid Biomineral dry weight [a] (mg)	Entrapment Yield [b] (%)	Protein load[b] ($mg_{protein}/mg_{material}$)	Ae ($U/mg_{protein}$)[c]
$Zn(COO)_2$ / 17	150	12.6	69	0.27	50.7
$ZnCl_2$ / 17	150	15.2	73	0.24	9.9
$Zn(COO)_2$ / 80	150	18	80	0.22	11.3
$Zn(COO)_2$ / 17	8	7.4	50	0.33	7.4

a) Hybrid biomineral dry weight was determined after drying the material at 80ºC. b) Entrapment yield **(%)** means the percentage of offered protein incorporated into the hybrid biomineral. c) Ae: Specific activity in IU x mg^{-1} of protein.

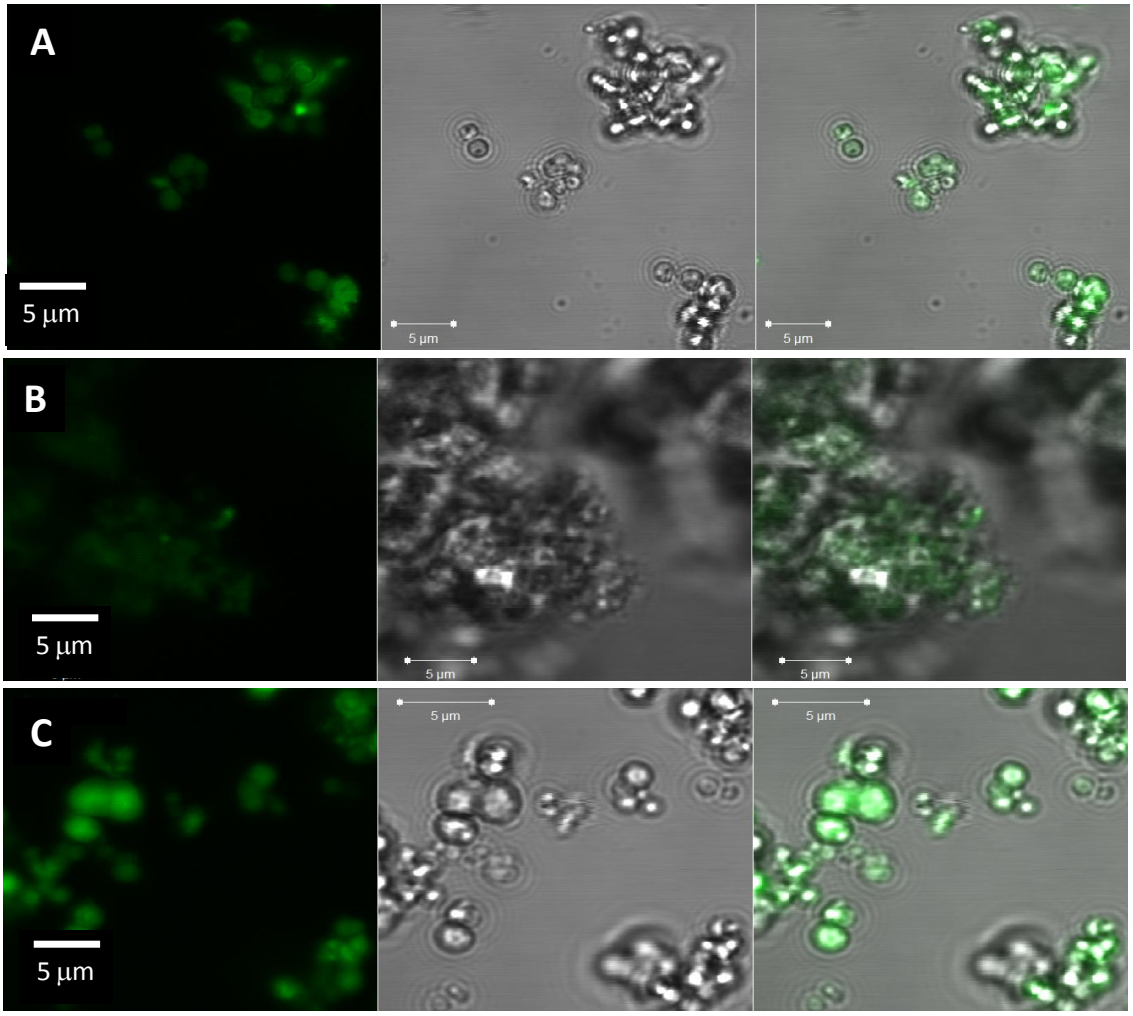

Figure S1. Confocal Laser scanning microscopy of different NFs made with fluoresceine-labelled TLL and (A) $Zn(CH_3COO)_2$, (B) $CaCl_2$ and (C) $CuSO_4$ in presence of saline phosphate buffer. The right column displays the fluoresceine fluorescence, the central column displays the brightfield and the left column is the overlay of both fluorescence and brightfield signals.

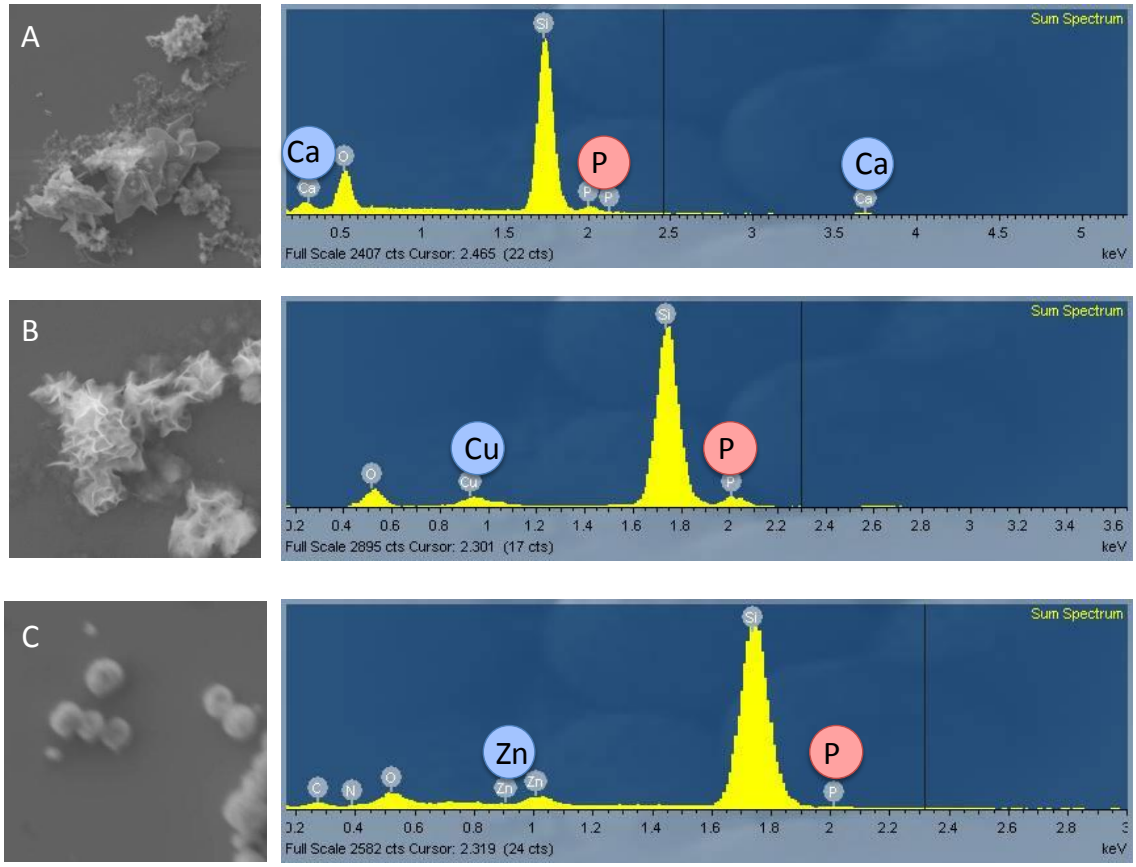

Figure S2. SEM-EDX images of different NFs made with TLL and (A) $Zn(CH_3COO)_2$, (B) $CaCl_2$ and (C) $CuSO_4$ in presence of saline phosphate buffer. The left column is the SEM images and the right colume are the total EDX spectrum of the SEM images.

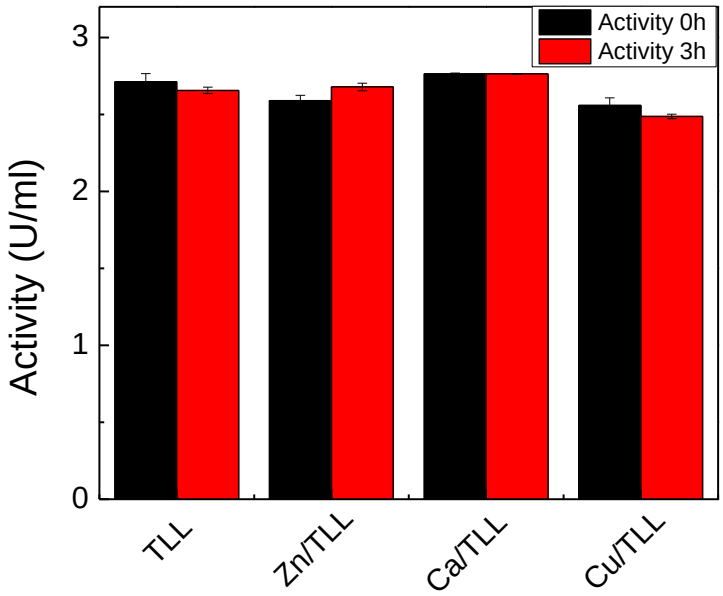

Figure S3. Activity to soluble enzyme with and without different metal salt under the biomineralization conditions but in buffer 25 mM Tris-H at pH 7 at 25°C

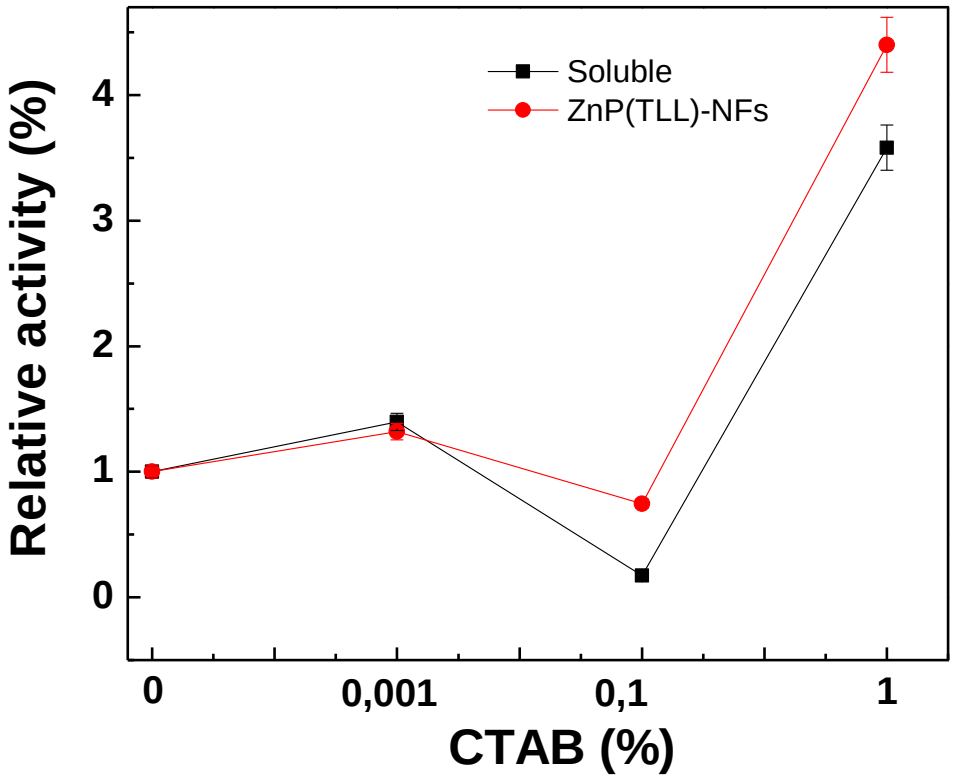

Figure S4. Hyperactivation profile of soluble TLL and different TLL-NFs in presence of CTAB**.** The activity was measured with p-NPB at 25° C and pH 7.

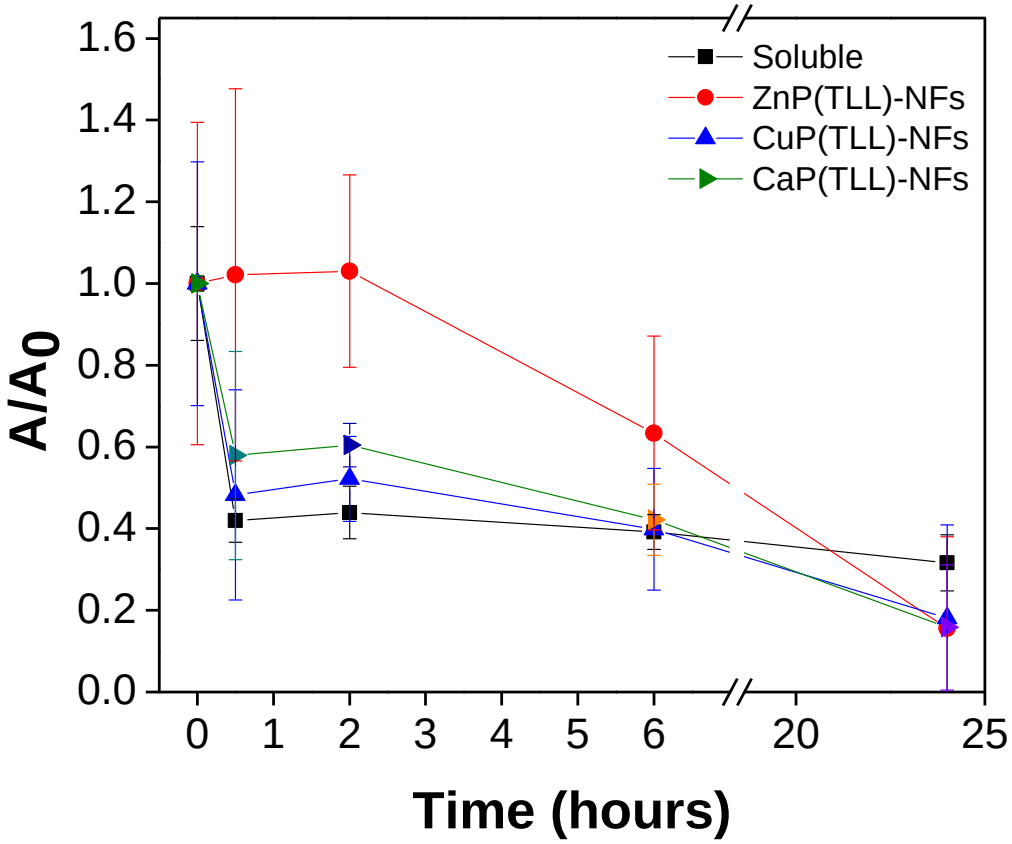

Figure S5. Thermal stability of TLL-nanoflowers at 65°C, pH 7 and phosphate buffer. The activity was monitored at different times using p-NPB as substrate

Table S3. Themal inactivation parameters of different preparations of TLL (soluble and NFs)

Biocatalyst	Mechanism[d]	$t_{1/2}$[a] (hours)	R^2[b]	SF[c]
Soluble	first-order Kinetic	0.3	1.00	--
ZnP(TLL)-NFs	first-order Kinetic	10.2	0.99	34
CaP(TLL)-NFs	Series-type	1.8	0.90	6
CuP(TLL)-NFs	Series-type	5.9	0.93	20

a)Half-life ($t_{1/2}$) values obtained by adjusting the experimental data to a classical first-order or series-type mechanism. b) The correlation coefficient (R^2). c)The stabilization factor (SF) is shown in each case$\tau_{1/2}$ means the half-life of the enzyme activity incubated at 65ºC and pH 7. d)The thermal inactivation along the time was fitted with two different inactivation mechanisms described by Henley et all[2,3] according to the following equations.

First order Kinetic mechanism:

$$\frac{\boldsymbol{A}}{\boldsymbol{A_0}} = \boldsymbol{exp}^{(-\boldsymbol{k_1}*\boldsymbol{t})}$$

Series-type mechanism:

$$\frac{\boldsymbol{A}}{\boldsymbol{A_0}} = \boldsymbol{\beta} + \left\{\mathbf{1} + \boldsymbol{\alpha}\frac{\boldsymbol{k_1}}{(\boldsymbol{k_2}-\boldsymbol{k_1})} - \boldsymbol{\beta}\frac{\boldsymbol{k_2}}{\boldsymbol{k_2}-\boldsymbol{k_1}}\right]\boldsymbol{exp}^{(-\boldsymbol{k_1}*\boldsymbol{t})}\right\} - \left\{(\boldsymbol{\alpha}-\boldsymbol{\beta})\left[\frac{\boldsymbol{k_1}}{(\boldsymbol{k_2}-\boldsymbol{k_1})}\right]\boldsymbol{exp}^{(-\boldsymbol{k_2}*\boldsymbol{t})}\right.$$

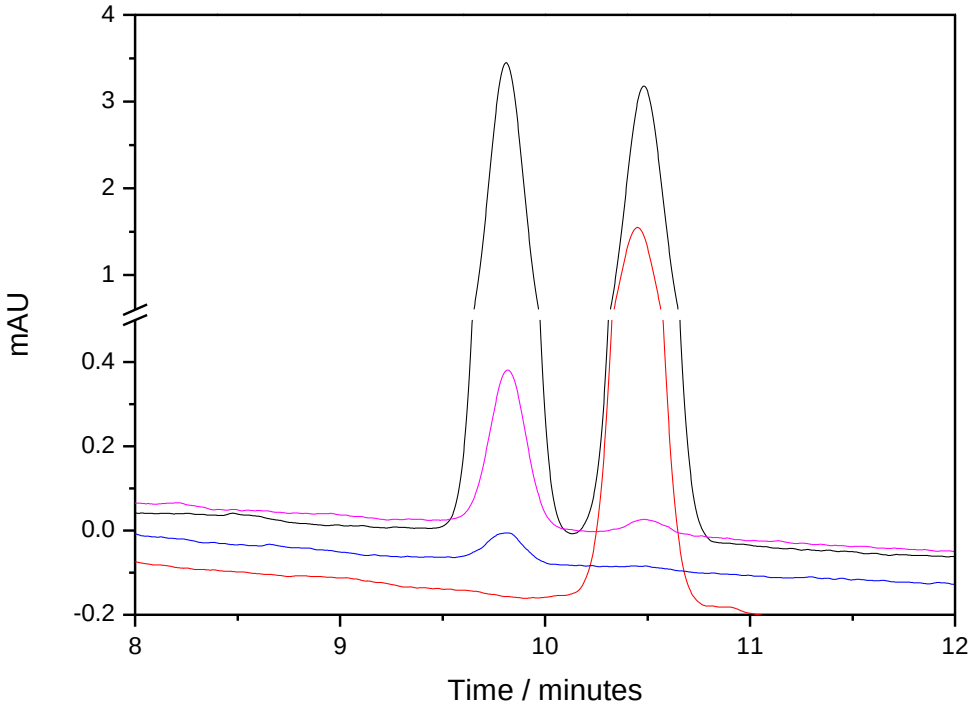

Figure S6. Chromatograms of kinetic resolution of rac-1-phenylethyl acetate catalyzed by different preparations of TLL. Standard of rac-1-phenyl ethanol (black). Standard of S-1-phenylethanol (red). Reaction catalyzed by ZnP(TLL)-NFs (purple). Reaction catalyzed by soluble TLL (blue).

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