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"The TYRP1-mediated protection of human tyrosinase activity does not involve stable interactions of tyrosinase domains."

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Supporting Information

Supporting Tables

Table S1. Purification of recombinant TYRP1tr from 10 g of larval biomass.

The total protein was determined by multiplying the protein concentration obtained from A_{260}/A_{280} by the sample total volume; TYRP1tr purity after each step of purification was obtained from the SDS-PAGE gels using UN-SCAN-IT gel ™ gel analysis software (Silk Scientific, Inc., UT).

Approximately 3-fold purification of TYRP1tr was obtained after IMAC, and ~7-fold purification was achieved by following GF chromatography. Finally, 3.6 mg of TRP1tr with a purity >95% was obtained from 10 g of larval biomass.

Table S2. Purification of four different combinations of coexpressed proteins, TYRtr/TYRP1tr, TYR/TYRP1, TYRtr/TYRP1, and TYR/TYRP1tr, from 10 g of larval biomass.

The total protein was determined by multiplying the protein concentration obtained from A_{260}/A_{280} by the sample total volume. Complex purity after each step of purification was obtained from the SDS-PAGE gels using UN-SCAN-IT gel ™ gel analysis software (Silk Scientific, Inc., UT).

Supporting Figures

Figure S1. TYR and TYRP1 homology at the active site.

Panel A shows that the active site of TYR contains two copper atoms (represented as orange spheres) that are coordinated by six histidines. In the active site of TYRP1 (**Panel B**), there are two atoms of zinc, which are depicted by purple spheres. Protein ribbons are colored in tan. **Panel C:** TYRP1 superimposed over TYR. TYR's ribbon is colored tan, while TYRP1's ribbon is depicted in blue. In the center, two metal atoms, shown as the orange spheres, are coordinated by six histidines. The inserted table shows the copper and zinc content in TYR and TYRP1 determined as described in the Materials and Methods.

Figure S2. Purification of TYRtr and TYRP1tr.

Panel A: Absorbance values at 280 nm recorded after two steps of purification using a ÄKTAxpress chromatography system. Chromatograms of TYRtr (black dashed line) and TYRP1tr (black solid line) eluted from a Sephacryl S-300 HR 16/60 column. **Panel B**: Pure TYRtr (black dashed line) and TYRP1tr (black solid line) eluted from a Superdex 200 Increase GL 10/300 column. The gray line shows the Bio-Rad GF standards. The inset shows pure TYRtr and TYRP1tr SDS-PAGE (top panel) and Western blots performed using anti-TYRP and anti-TYR antibodies (middle and bottom panels, respectively). **Panel C**: Sedimentation equilibrium of TYRP1tr. The protein concentration profile of absorbance (280 nm) versus radial distance is indicated. The black line shows the calculated fit for the monomer. Open circles represent experimental values. The top panel shows the residuals of a curve fitted to the data points. The sedimentation equilibrium profile of TYRtr has been published previously (Dolinska et al., 2014).

Figure S3. Parameters determined from the TYRtr diphenol oxidase kinetic profiles.

Blue line: the initial velocity (V_0) calculated from the slope of the reaction time curve. Green line: a maximum of dopachrome concentration measured in μM using the Beer-Lambert Law, $A=\varepsilon bc$, where A is absorbance; ε is the extinction coefficient for dopachrome at 475 nm (3700 M^{-1} cm^{-1}); b is the path length of the well in which the sample was contained (cm); and c is the concentration of TYRtr in μM. Gray line: time when maximum dopachrome formed, shown in minutes. Red line: time (min) when melanin-like precipitation began.

Figure S4. Purification of intramelanosomal domains of TYR and TYRP1 individually expressed in larval biomasses.

Panel A: Absorbance values at 280 nm recorded after two steps of purification using a ÄKTAxpress chromatography system. Chromatography of TYRP1tr (black solid line), TYRtr (black dashed line), and a 1:1 mixture of larva-expressed TYRtr and TYRP1tr (green line) eluted from a Sephacryl S-300 HR 16/60 column. **Panel B**: SDS-PAGE (top panel) and Western blot with anti-TYRP1 (middle panel) and anti-

TYR (bottom panel) for a 1:1 mixture of larva-expressed TYRtr and TYRP1tr (green frame), TYRP1tr alone (black solid frame), and TYRtr alone (black dashed frame).

Figure S5. Purification of TYR and TYRP1 individually expressed by larval biomasses.
Panel A: IMAC using a GE Healthcare His-Trap 5 ml column **Panel B**: GF performed with a Superdex 200 Increase GL 10/300 column. Insets in both panels show the fractions corresponding to SDS-PAGE (top panel) and Western blots using anti-TYRP1 (G 17, middle panel) and anti-TYR antibodies (T311, bottom panel). Numbers at the top display the protein ladder and number of collected fractions.

Figure S6. Purification of mixtures of two tyrosinases coexpressed in larvae.
IMAC (using a GE Healthcare His-Trap 5 ml column) and GF (using Sephacryl S-300 HR 16/60 and Superdex 200 Increase GL 10/300 columns) of TYRtr/TYRP1tr (**Panel A**), TYRtr/TYRP1 (**Panel B**), TYR/TYRP1tr (**Panel C**), and TYR/TYRP1 (**Panel D**). The insets show the fractions corresponding to SDS-PAGE (top panel) and Western blots using anti-TYRP1 (G 17, middle panel) and anti-TYR antibodies (T311, bottom panel). Numbers at the top display the protein ladder and number of collected fractions.

Figure S7. Electrostatic effects from surface properties of human tyrosinase and tyrosinase-like protein 1 as a function of pH. **Panels A-B** show electrostatic potential surfaces (calculated at pH 5.5 and pH 7.2) around the homology model of human TYR obtained as described in the Methods section. **Panels C-D** show the electrostatic potential profiles calculated at pH 5.5 and pH 7.2 for the human TYRP1 structure obtained from the RCSB database (File: 5m8l). The electrostatic field around the protein structure was calculated using the Poisson-Boltzmann Solver incorporated in the molecular graphics, modeling and simulation program YASARA (http://www.yasara.org/). Electrostatic potential was visualized using a contour procedure at the 1 kJ/mol level. The maximum absolute potential was 10 kJ/mol, and the grid resolution was 0.5 Å.

Figure S8. **Effect of calcium and low temperature *in vitro*.** Calcium presence show no change in homo- and hetero-association of TYRtr and TYRP1tr. The association was measured in the presence (**Panel A**) or the absence (**Panel B**) of calcium. The pure proteins and their mixture at 1:1 ratio objected to the size-exclusion chromatography using the Superdex 75 10/300 column of 10 mM $CaCl_2$ in 10 mM Tris, pH 7.2. The graphs show the chromatography profiles of TYRtr (black dashed line), TYRP1tr (black solid lane), and TYRtr/TYRP1tr mixtures at a ratio of 1:1 (red line). Gray profile in each panel corresponds to the Bio-Rad GF standards: thyroglobulin (670 kDa), γ-globulin (158 kDa), ovalbumin (44 kDa), and myoglobulin (17 kDa). Inserts show the particle size distribution by intensity of TYRtr (black dashed line), TYRP1tr (black solid lane), and TYRtr/TYRP1tr mixtures at a ratio of 1:1 (red line). **Panel C**: The

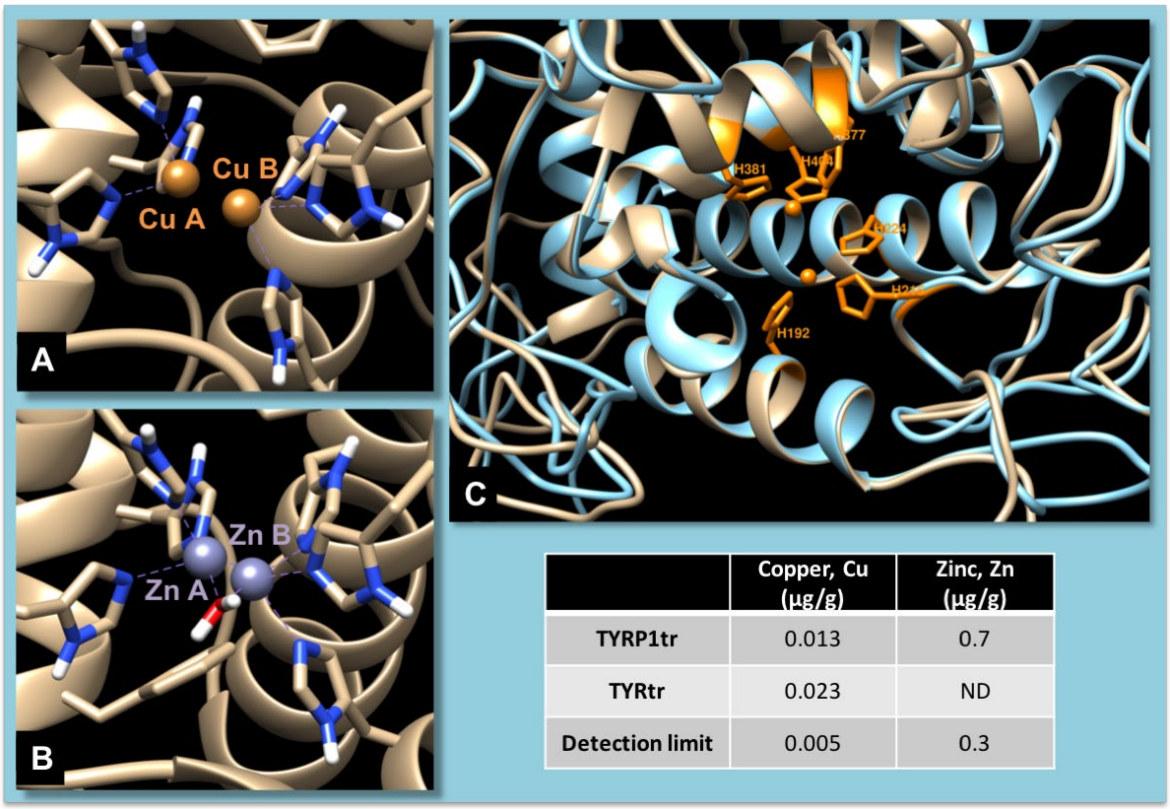
Table shows the hydrodynamic diameter of suspended particles of TYRtr, TYRP1tr, and TYRtr/TYRP1tr 1:1 calculated from the dynamic light scattering. The hydrodynamic diameter corresponds to the diameter of 'equivalent sphere' for the protein atomic model covered by a single layer of water. Dynamic light scattering was measured using Litesizer 500 (Anton Paar USA, VA). **Panel D:** Temperature dependence of diphenol oxidase activity of TYRtr show no tyrosinase enzymatic activity at 4°C. The activity of 1.5 mM L-Dopa with (+) and without (-) TYRtr was measured at 3 temperatures (4°C, 25°C, and 37°C) in 10 mM in sodium phosphate buffer. The inserts above each bar correspond to the 30 min sample incubations at corresponding temperatures and visibly show the changes in colored reactions with increasing dark brown color. Endpoint spectrophotometer readings in (+) tubes obtained after 30 minutes of reaction time are shown by vertical bars (dopachrome concentration).

	Total protein (mg)	TYRP1tr purity (%)
Lysate	2409 ± 289	13.24 ± 0.13
IMAC	15.29 ± 1.19	53.52 ± 0.62
GF1	4.61 ± 1.76	81.20 ± 1.81
GF2	3.60 ± 1.06	95.50 ± 1.30

Table S1.

	Total protein (mg)					Complex purity (%)				
	Lysate	**IMAC**	**GF1**	**GF2**	**GF3**	**Lysate**	**IMAC**	**GF1**	**GF2**	**GF3**
TYRtr/TYRP1tr	3047	14.88	2.67	1.68	-	8.06	43.30	85.04	96.96	-
TYR/TYRP1	2500	18.48	7.49	1.65	0.23	8.20	48.46	51.70	73.05	86.12
TYRtr/TYRP1	2773	19.60	2.64	0.64	0.12	7.35	62.77	89.97	91.38	95.62
TYR/TYRP1tr	2625	18.80	2.90	1.20	0.19	8.55	68.24	73.40	89.41	93.56

Table S2.



	Copper, Cu (µg/g)	Zinc, Zn (µg/g)
TYRP1tr	0.013	0.7
TYRtr	0.023	ND
Detection limit	0.005	0.3

Figure S1.

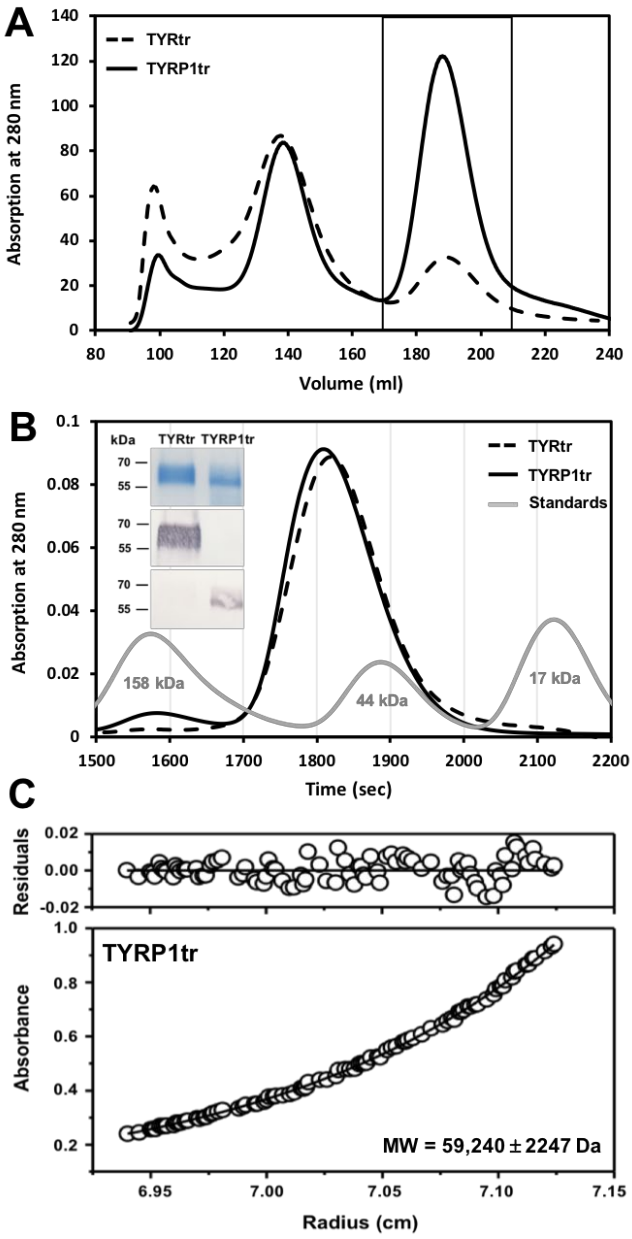

A
TYRtr
TYRP1tr
Absorption at 280 nm
Volume (ml)
B
kDa
TYRtr TYRP1tr
70
55
Standards
158 kDa
44 kDa
17 kDa
Absorption at 280 nm
Time (sec)
C
Residuals
TYRP1tr
Absorbance
MW = 59,240 ± 2247 Da
Radius (cm)

Figure S2.

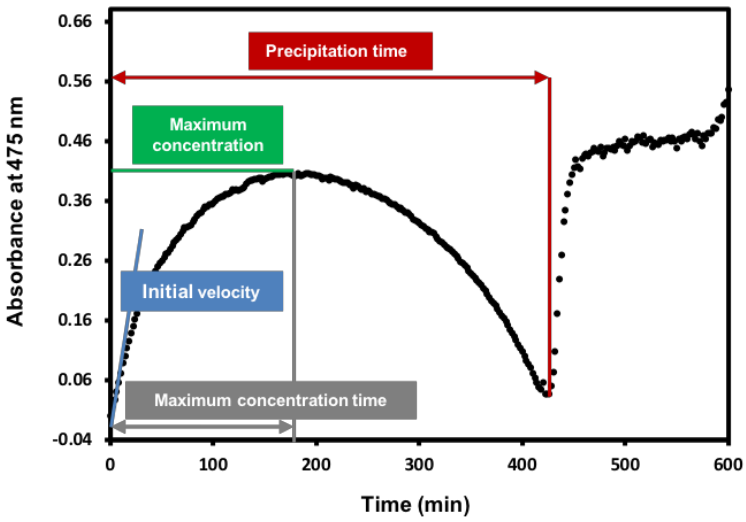

Precipitation time
Maximum concentration
Initial velocity
Maximum concentration time
Absorbance at 475 nm
Time (min)
0.66
0.56
0.46
0.36
0.26
0.16
0.06
-0.04
0
100
200
300
400
500
600

Figure S3.

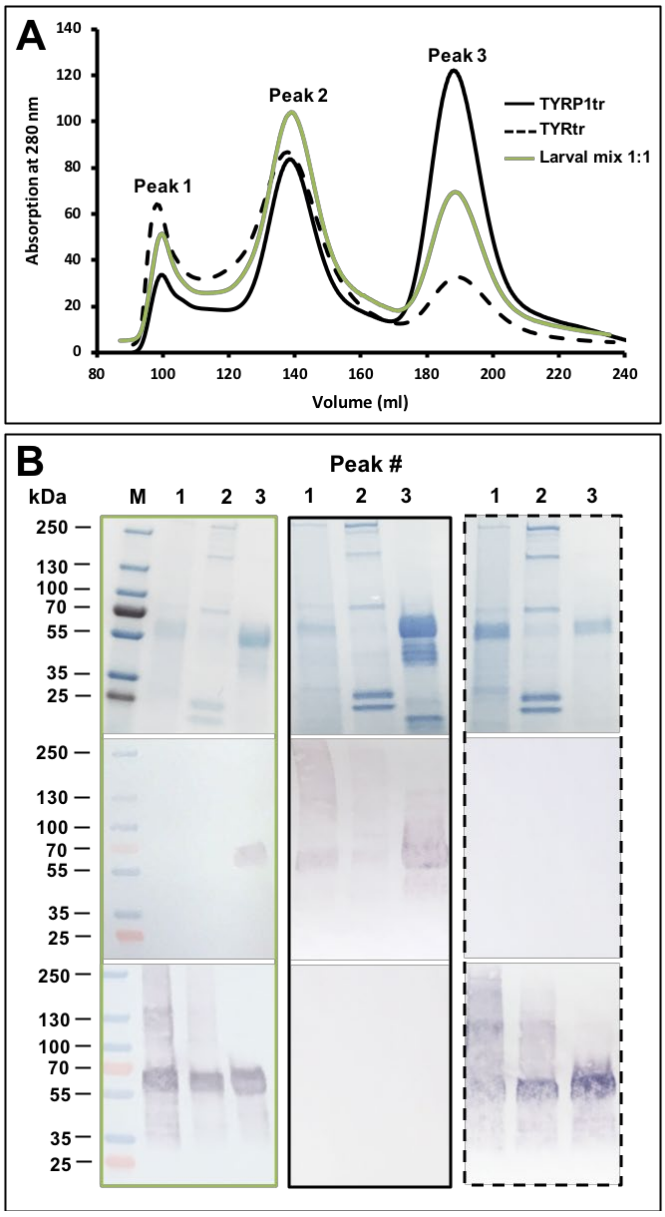

A
Absorption at 280 nm
Volume (ml)
Peak 1
Peak 2
Peak 3
TYRP1tr
TYRtr
Larval mix 1:1
B
Peak #
kDa
M
1
2
3
250
130
100
70
55
35
25

Figure S4.

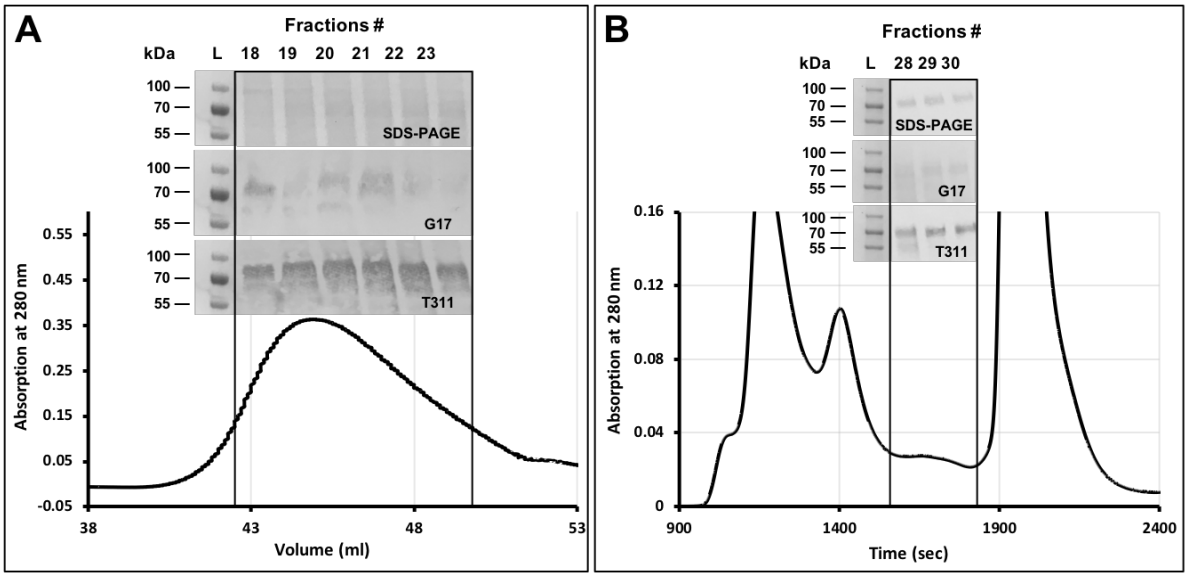

A
Fractions #
kDa
L
18
19
20
21
22
23
100
70
55
SDS-PAGE
G17
T311
Absorption at 280 nm
0.55
0.45
0.35
0.25
0.15
0.05
-0.05
38
43
48
53
Volume (ml)
B
Fractions #
kDa
L
28 29 30
SDS-PAGE
G17
T311
0.16
0.12
0.08
0.04
0
900
1400
1900
2400
Time (sec)

Figure S5.

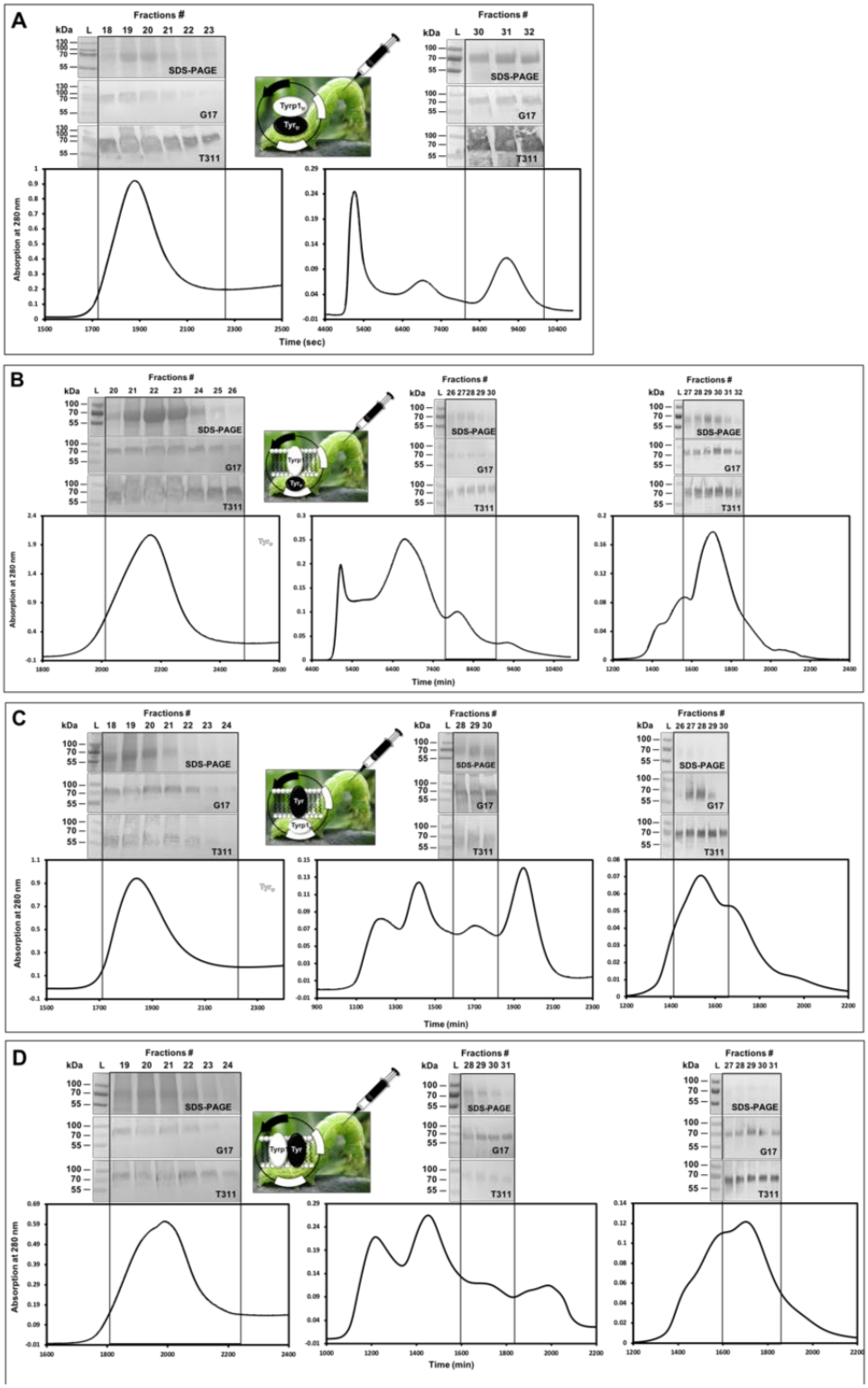

A
Fractions #
kDa L 18 19 20 21 22 23
SDS-PAGE
G17
T311
Tyrp1
Tyr
Fractions #
kDa L 30 31 32
SDS-PAGE
G17
T311
Absorption at 280 nm
Time (sec)
B
Fractions #
kDa L 20 21 22 23 24 25 26
SDS-PAGE
G17
T311
Tyrp
Tyr
Fractions #
kDa L 26 27 28 29 30
SDS-PAGE
G17
T311
Fractions #
kDa L 27 28 29 30 31 32
SDS-PAGE
G17
T311
Absorption at 280 nm
Time (min)
C
Fractions #
kDa L 18 19 20 21 22 23 24
SDS-PAGE
G17
T311
Tyr
Tyrp1
Fractions #
kDa L 28 29 30
SDS-PAGE
G17
T311
Fractions #
kDa L 26 27 28 29 30
SDS-PAGE
G17
T311
Absorption at 280 nm
Time (min)
D
Fractions #
kDa L 19 20 21 22 23 24
SDS-PAGE
G17
T311
Tyrp1
Tyr
Fractions #
kDa L 28 29 30 31
SDS-PAGE
G17
T311
Fractions #
kDa L 27 28 29 30 31
SDS-PAGE
G17
T311
Absorption at 280 nm
Time (min)

Figure S6.

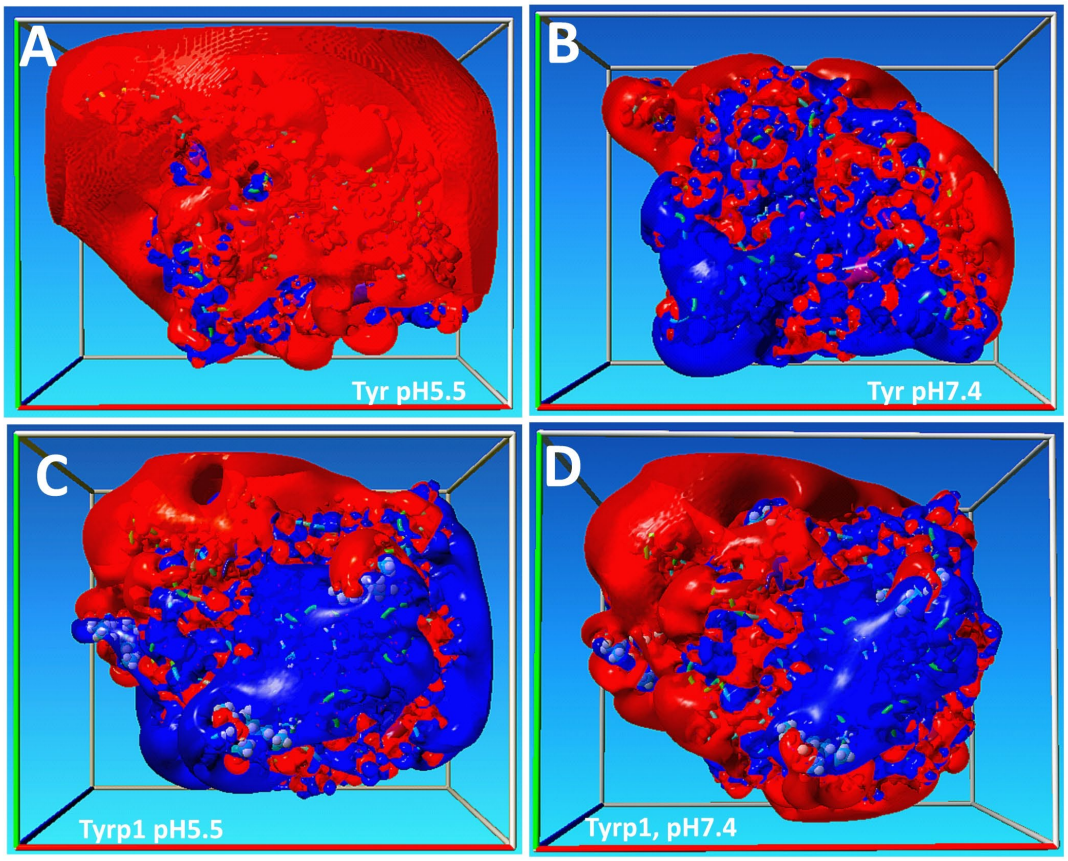
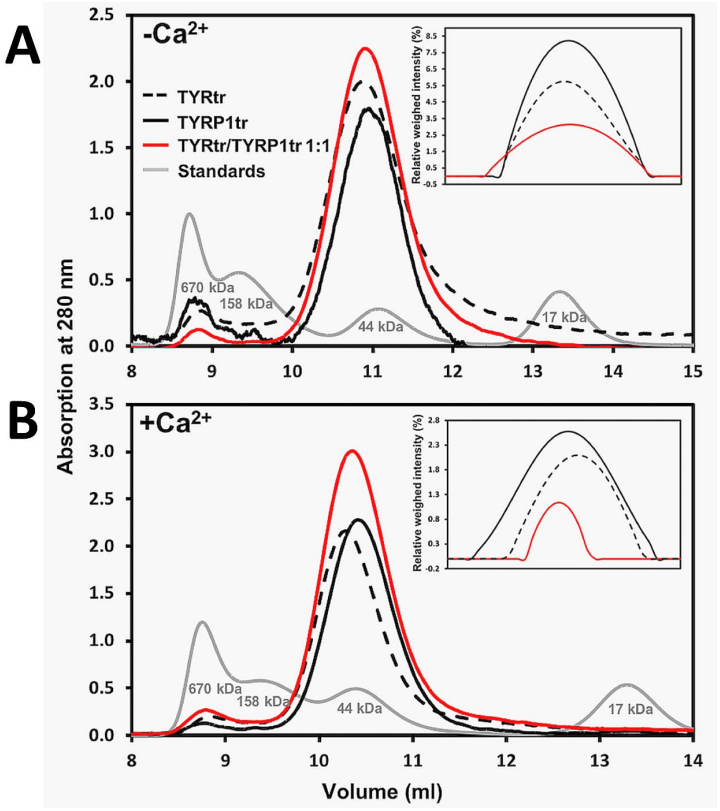


Figure S7.



C

	Hydrodynamic diameter (nm)		
Calcium presence	TYRtr	TYRP1tr	TYRtr/TYRP1tr 1:1
-	8.31 ± 0.57	8.24 ± 0.37	8.21 ± 0.26
+	8.08 ± 1.27	7.18 ± 1.54	7.04 ± 0.19

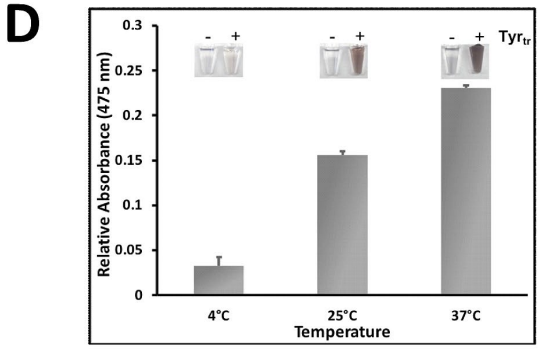


Figure S8.