Supporting Information for

Characterization of the artemisinin binding site for translationally controlled tumor protein (TCTP) by biorthogonal click chemistry

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1. Biological experimental procedures

1.1. Expression and purification of *Pf*TCTP

The genes of *P. falciparum* translationally controlled tumor protein (*Pf*TCTP) and its mutations were synthesized and cloned into pET21a expression vectors to obtain His6-tag fusions by GENEWIZ Inc. (Suzhou, China). The resulting plasmids were transformed into BL21(DE3) *E. coli* which is optimized for expression of desire genes. Luria-Bertani (LB) medium (1000 mL) containing 50 μg/mL ampicillin (LB_{amp50}) was inoculated with 500 μL of an overnight culture corresponding to each candidate gene and a vector control. These were grown to an OD_{600} of approximately 0.6. Recombinant protein expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.3 mM. Cultures were grown for further 20 h at 16°C with shaking. At 20 h, 1.0 mL of culture was collected from each culture for SDS polyacrylamide gel electrophoresis (PAGE) analysis and the remaining was pelleted at 8000g for 15 min at 4°C. The samples for SDS-PAGE were resuspended in 100-200 μL of cracking buffer (50 mM Tris-HCl, pH 7.4, 1% v/v 2-mercaptoethanol, 1% SDS w/v, 6 M urea) depending on the pellet volume. A 10% SDS-PAGE gel containing 5.0 μL of each resuspended sample was run. The recombinant protein was stained using the coomassie blue.

The bacterial pellets were resuspended in 50 mL of resuspension buffer (0.2% Triton X-100 and 50 mM Tris-HCl, pH 7.4). Lysozyme was added to a final concentration of 100 μg/mL and the samples were incubated at 0°C for 20 min. The cell lysate was centrifuged at 18,000 g for 15-30 min at 4°C and the supernatant was collected. The clear supernatant was loaded onto the pre-equilibrated Ni-NTA column and washed with 10 column volume of wash buffer (50 mM Tris-HCl, pH 7.4). The protein was gradually eluted with the elution buffer (50 mM Tris-HCl, pH 7.4, gradually increasing the concentration of the imidazole, 0-300 mM). The fractions contained the protein detected by SDS-PAGE were collected and dialyzed against proper buffer.

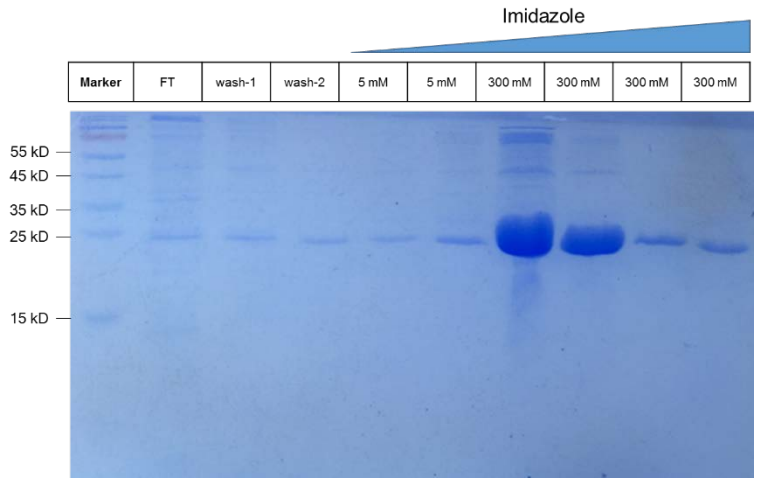

Figure S1. The purification of wild-type *Pf*TCTP. **FT**: flow through fraction; wash-1 and wash-2: eluted fractions by washing buffer; **5 mM**: eluted fractions by elution buffer (50 mM Tris-HCl pH 7.4, 5 mM imidazole); **300 mM**: eluted fractions by elution buffer (50 mM Tris-HCl, pH 7.4, 300 mM imidazole).

1.2. Competitive labeling of recombinant wildtype *Pf*TCTP protein

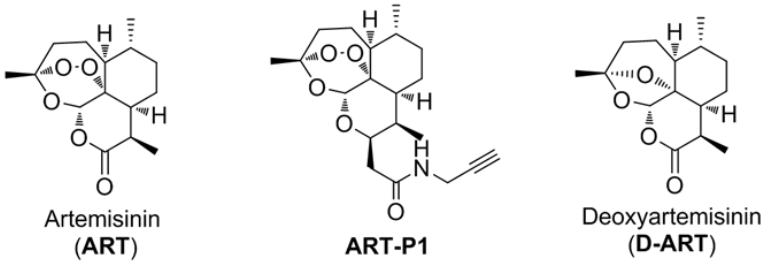

Figure S2. Structures of artemisinin (**ART**), artemisinin activity-based probe (**ART-P1**) and artemisnin inactive analog deoxyartemisinin (**D-ART**).

Recombinant *Pf*TCTP dissolved in PBS (0.1 mg/mL) was incubated with 100 μM of **ART** and **D-ART**, respectively, at room temperature for 1 h. **ART-P1** (10 μM) and hemin (10 μM) were added sequentially to the samples and incubated at room temperature for 2 h. 1% SDS (w/v) was added to each sample and click reaction was performed as below: for each reaction, 20 μL of protein samples were added freshly prepared 0.25 μL each of TAMRA-N_3 (10 mM stock in DMSO, Lumiprobe), $CuSO_4$

(100 mM stock in H_2O), THPTA (Tris(3-hydroxypropyltriazolylmethyl) amine, 10 mM stock in H_2O, Sigma) and ascorbic sodium (NaVc, 100 mM stock in H_2O, Sigma). The samples were incubated at room temperature for 1 h, added with SDS sample loading buffer, applied to SDS-PAGE and imaged by FUJIFILM FLA 9000 plus DAGE fluorescence scanner.

1.3. Probe/hemin-concentration dependent labeling of *Pf*TCTP protein

Recombinant *Pf*TCTP dissolved in PBS (0.1 mg/mL) were added with 10 μM of hemin and various concentrations of **ART-P1**, or 10 μM of **ART-P1** and various concentrations of hemin as indicated in Figure 2B and 2C. The samples were incubated at room temperature for 2 h and the following click reaction and fluorescence scan were performed same as above.

1.4. Block/recovery of labeling of *Pf*TCTP protein

Recombinant *Pf*TCTP dissolved in PBS (0.1 mg/mL) were added with 20 mM iodoacetamide (IAA, Sigma) or N-ethylmaleimide (NEM, Sigma). After 1-h incubation at room temperature, 1.0 mM of NaVc was added. Then **ART-P1** (10 μM) and hemin (10 μM) were added to the samples and incubated at room temperature for another 2 h. The following click reaction and fluorescence scan were performed same as above.

1.5. Labeling of *Pf*TCTP mutant proteins

Recombinant *Pf*TCTP dissolved in PBS (0.1 mg/mL) were incubated with **ART-P1** (10 μM) and hemin (10 μM) in the absence and presence of NaVc (100 μM) at room temperature for 2 h. The following click reaction and fluorescence scan were performed same as above.

2. Mass spectrometry analysis of ART-P1-TCTP conjugates

2.1. Enrichment of ART-P1 alkylated peptides

100 μg of *Pf*TCTP dissolved in PBS (5.0 mg/mL, ~250 μM) was incubated with **ART-P1** (10 μM), hemin (10 μM) and NaVc (10 μM) at room temperature for 3 h. The samples were then subjected to click reaction with acid-cleavable biotin-azide (**DADPS**, 500 μM), $CuSO_4$ (1.0 mM), THPTA (100 μM) and NaVc (1.0 mM). The samples were precipitated with CH_3OH (600 μL) / $CHCl_3$ (150 μL) / H_2O (300 μL) sequentially and vortexed. After centrifuging at 14,000 g for 3 minutes, the protein disk was washed twice with CH_3OH (500 μL), air-dried and re-dissolved in 200 μL of click buffer (50 mM HEPES pH 8.0, 1% SDS) by sonication. 50 μL of streptavidin-sepharose (GE Healthcare) beads were added to each sample and incubated at room temperature with continuous rotation for 1 h. The beads were washed with PBS, 1% SDS × 3, 4 M Urea × 3, PBS × 3, 50 mM NH_4HCO_3 × 3. The bounded proteins were eluted by 5% HCOOH overnight at room temperature. The eluent was neutralized by NH_4HCO_3 (100 mM) and concentrated by ultrafiltration. The proteins were reduced in 2.0 mM DTT at 56°C for 30 min, alkylated in 10 mM iodoacetamide in darkness for 1 h, and desalted by Pierce C18 Spin Columns (Thermo Scientific). Then the proteins were digested with 5.0 μg of trypsin (Promega) reconstituted in 200 μL of 50 mM NH_4HCO_3 for 16 h at 37 °C. The digests were evaporated to dryness on a SpeedVac and resuspended in 30 μL of formic acid/acetonitrile/H_2O (v:v:v = 0.5%/2%/97.5%) with sonication.

2.2. LC-MS/MS

After syringe filtration through 22 μm filters, the clear solution were subjected to nano LC-MS/MS separation. A volume of 3.0 μL of each sample was desalted by loading on a Thermo C18 PepMap100 precolumn (300 μM × 5 mm) and eluted on a Thermo Acclaim PepMap RSLC analytical column (75 μM × 15 cm). Mobile phase A (0.1% formic acid in H_2O) and mobile phase B (0.1% formic acid in acetonitrile) were used to establish the 120-min gradient elution process. The flow rate was 0.3 μL/min. Peptides were then analyzed in a Q-Exactive proteomic mass spectrometer (Thermo Scientific) with a

data-dependent manner, with automatic switching between MS and MS/MS scans using a top-10 method. MS spectra were acquired at a resolution of 70,000 with a target value of 3×10^6 ions or a maximum integration time of 50 ms. The scan range was limited from 180 to 6000 *m/z*. Peptide fragmentation was performed via higher-energy collision dissociation (HCD) with the energy set at 32 NCE. The MS/MS spectra were acquired at a resolution of 17,500 with a target value of 1×10^5 ions or a maximum integration time of 60 ms. The fixed first *m/z* was 120, and the isolation window was 2.0 *m/z*. Peptide sequences (and hence protein identity) were determined by matching protein databases with the acquired fragmentation pattern by the software program, Sequest, filtered to a 1% peptide FDR via the target-decoy approach, using a linear discriminant function to score each peptide based on parameters such as Xcorr, DCn, and precursor mass error. The precursor-ion mass tolerance was set to 10 ppm. and the fragment-ion mass tolerance was 0.02 Da.

2.3. Mass data processing

Data processing was performed using Proteome Discoverer 1.4 software (Thermo Scientific) and peptide sequences were determined by matching protein databases with the acquired fragmentation pattern by SEQUEST HT algorithm. The precursor mass tolerance was set to 10 ppm and fragment ion mass tolerance to 0.02 Da. One missed cleavage site of trypsin was allowed. Model A_1 (Figure S6, any amino acids), Model A_2 (Figure S6, any amino acids), Carbamidomethyl (C), Oxidation (M), and deamination (N, Q) were used as variable modifications. All spectra were searched against protein sequence of recombinant *Pf*TCTP using a target false discovery rate (FDR) of 1%.

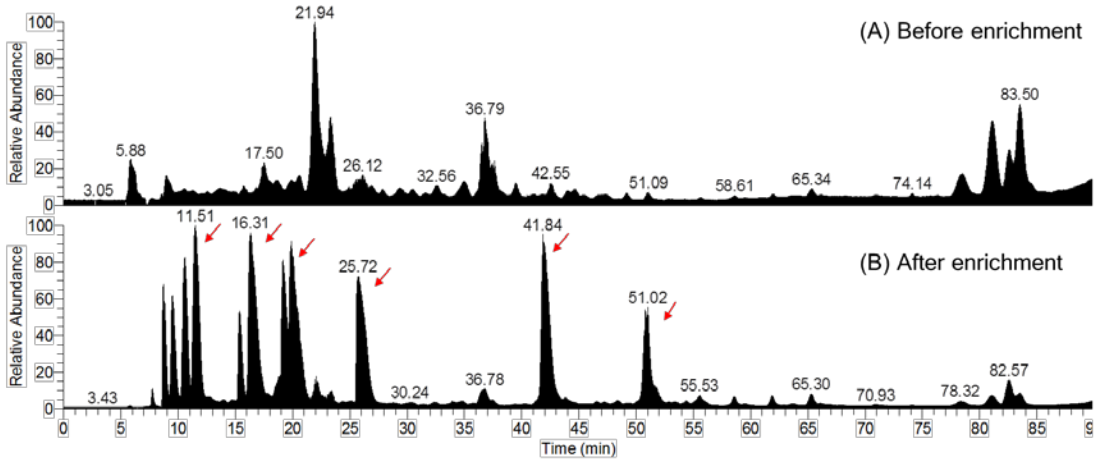

Figure S3. The total ion chromatography (TIC) spectra of the samples (A) before and (B) after capture-and-release by **DADPS**.

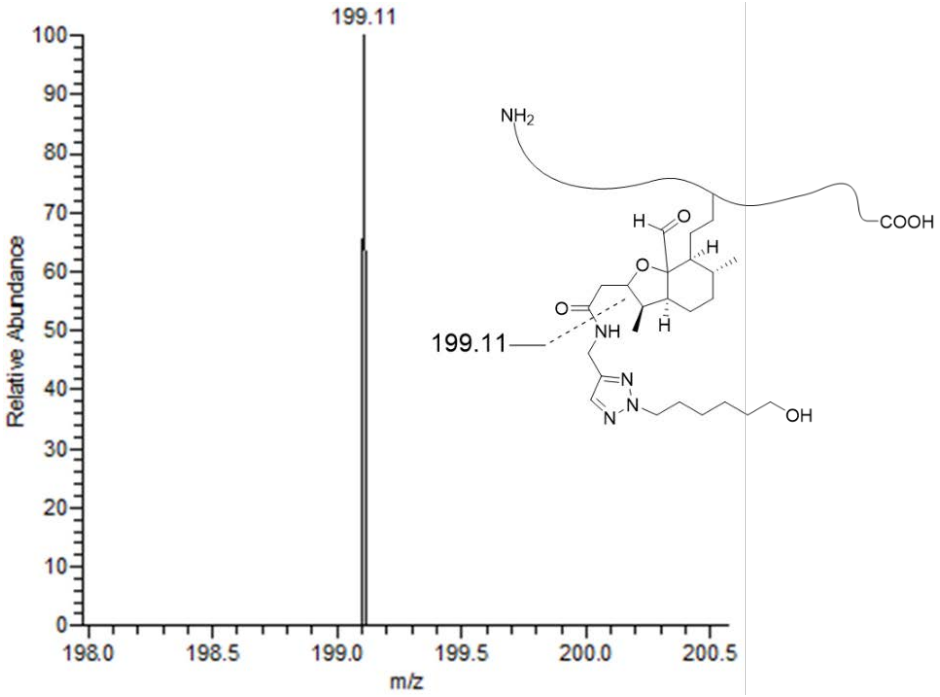

Figure S4. The MS2 spectrum the diagnostic fragment ions (DFI) fragment ion of **ART-P1**-alkylated peptide.

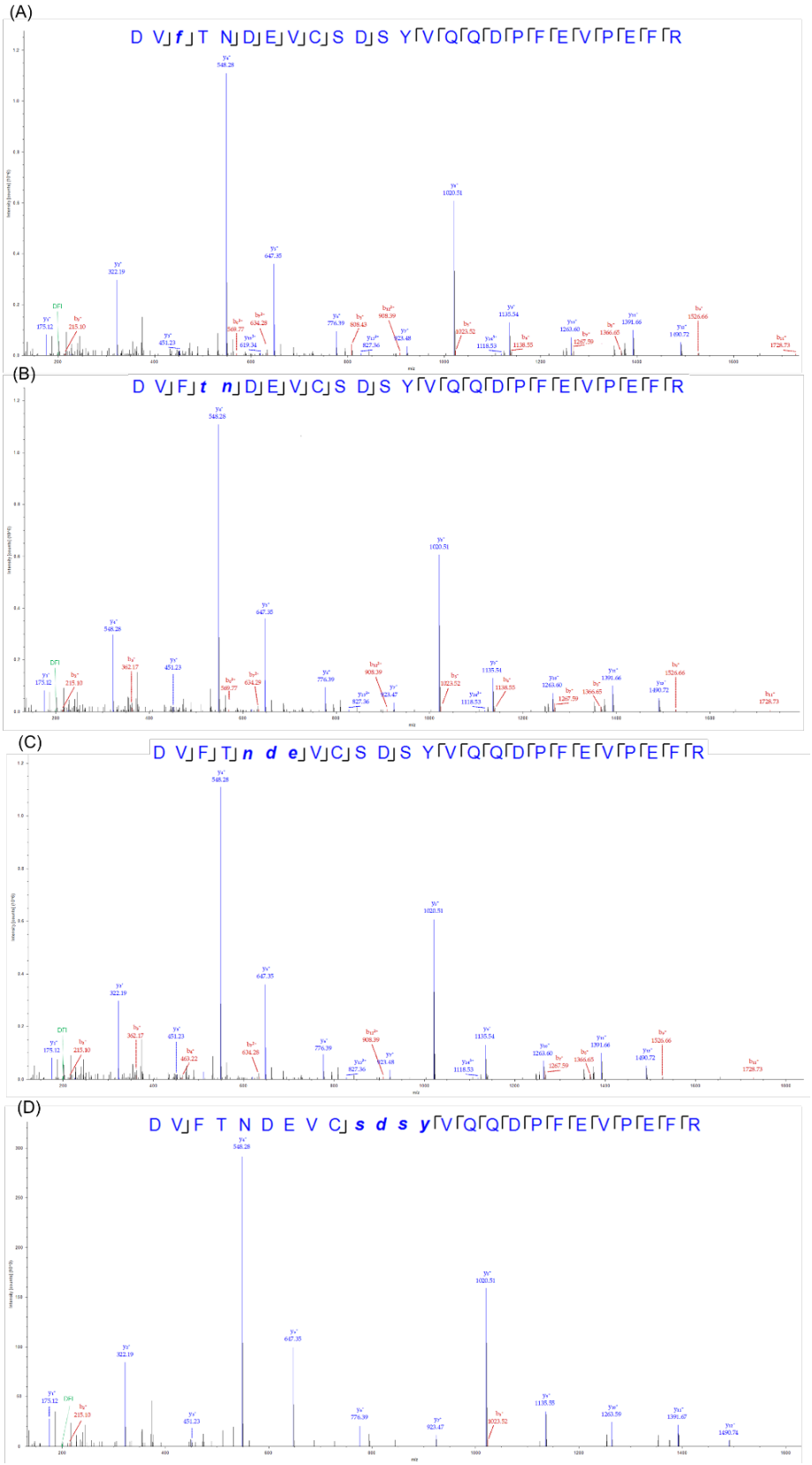

Figure S5. Representative HCD spectra of **ART-P1** alkylated peptide. Annotated modification sites were marked in lower-case and italic. (A) Phe12 was modified; (B) Thr13 and Asn14 could be modified; (C) Asn14, Asp15 and Val16 could be modified; (D) Ser19, Asp20, Ser21 and Tyr22 could be modified.

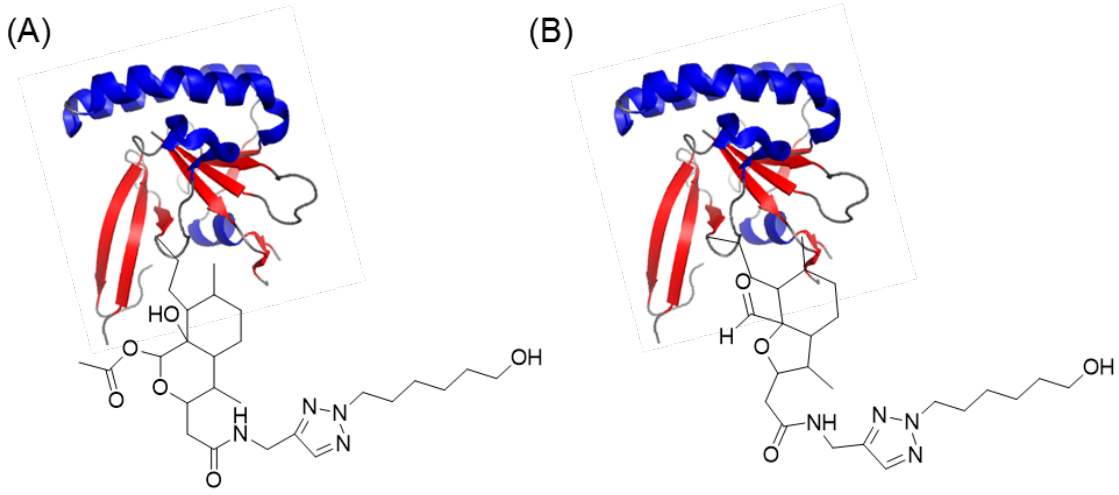

Figure S6. Two models of probe-adducted TCTP complex after acid cleavage. (A) Model A_1 Δmass = 506.3104; (B) Model A_2 Δmass = 446.2893 (A_1 - acetate).

Table S1. Fragment ions of peptide (DVFTNDEVCSDSYVQQDPFEVPEFR), red and blue numbers indicated fragments which were matched with calculated mass of corresponding fragments (D: Deamination; C: Carbamidomethyl; ND: not detected).

#1	*b*- series ions		Seq.	*y*- series ions		#2
	Unmodified	Modified		Unmodified	Modified	
1	116.03	ND	D			25
2	215.10	ND	V	2850.26	ND	24
3	362.17	808.46 $[b_3+A_2]^+$	F	2751.19	1085.50$[y_{23}+A_2]^{3+}$	23
4	463.22	ND	T	2604.12	ND	22
5	577.26	1023.55$[b_5+A_2]^+$	N	2503.07	ND	21
6	692.29	1138.58$[b_6+A_2]^+$	D	2389.03	965.78$[y_{20}+D+A_2]^{3+}$	20
7	821.33	1267.62$[b_7+A_2]^+$	E	2274.00	ND	19
8	920.40	1366.69$[b_8+A_2]^+$	V	2144.96	ND	18
9	1023.41	1529.72$[b_9+A_1]^+$	C	2045.89	ND	17
10	1110.44	ND	S	1942.88	ND	16
11	1225.47	1728.78$[b_{11}+A_2]^+$	D	1855.85	ND	15
12	1312.50	908.41$[b_{12}+C+A_2]^{2+}$	S	1740.82	ND	14
13	1475.56	ND	Y	1653.79	720.58$[y_{13}+A_2]^{3+}$	13
14	1574.63	ND	V	1490.73	ND	12
15	1702.69	ND	Q	1391.66	ND	11
16	1830.75	ND	Q	1263.60	ND	10
17	1945.78	ND	D	1135.54	ND	9
18	2042.83	ND	P	1020.51	ND	8
19	2189.90	ND	F	923.46	ND	7
20	2318.94	ND	E	776.39	ND	6
21	2418.01	ND	V	647.35	ND	5
22	2515.06	ND	P	548.28	ND	4
23	2644.10	ND	E	451.23	ND	3
24	2791.17	ND	F	322.19	ND	2
25			R	175.12	ND	1