

Supplementary Materials for

Zinc-dependent oligomerization of *Thermus thermophilus* trigger factor

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Table S1

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Table S1. The primers and templates for plasmid construction.

Primer	Oligo sequence (5' to 3')	PCR template	Constructed plasmid
1	CATCACGTGGCGGAGATCCTG	<i>Tt</i> TF gene	<i>His</i> ₆ - <i>Tt</i> TF
2	GAACAGAACTTCCAGTCAGCCCAGGG	segment	
3	CCGCCACGTGATGATGATGAT	pCold	
4	TGACTGGAAGTTCTGTTC	<i>Ec</i> TF ^{RBD} vector	
5	CATCATCACATGGAAGTTAAAGAACTTG	<i>Tm</i> TF gene	<i>His</i> ₆ - <i>Tm</i> TF
6	CCGCGGATTAGTTGTCCTCCTTCCC	segment	
7	GACAACTAATCCGCGGGTCTGGAA	pCold	
8	AACTTCCATGTGATGATGATGATGATG	<i>Ec</i> TF ^{RBD} vector	
9	GAAAACCTGTATTTTCAGGGTGTGGCGGAGATCCTGGAG	<i>His</i> ₆ - <i>Tt</i> TF	<i>His</i> ₆ -TEV- <i>Tt</i> TF
10	ACCCTGAAAATAAGGTTTTCTGTGATGATGATGATG		
11	GAAAACCTGTATTTTCAGATGCAAGTTTCAGTTGA	<i>His</i> ₆ - <i>Ec</i> TF	<i>His</i> ₆ -TEV- <i>Ec</i> TF
12	CTGAAAATACAGGTTTTCTGTGATGATGATGATGATG		
13	CCCCGAGTGACTGGAAGTTCTGTTC	<i>His</i> ₆ -TEV- <i>Tt</i> TF	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{RBD}
14	TCCAGTCACTCGGGGTAGTTCTCCAC		
15	TCAGGGTCCCGAGGTGAGGCTTCCC	<i>His</i> ₆ -TEV- <i>Tt</i> TF	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{PPD-SBD}
16	ACCTCGGGACCCTGAAAATACAGGTT		
17	CTACGCCGAGCTGGACGAGGAGTTC	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{PPD-SBD}	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{SBD}
18	TCCAGCTCGGCGTAGCGCTGGCGGAG		
19	GGTAGTGGCAGCGGAGAGCTGGACGAGGAGTTC	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{SBD}	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{SBD}
20	TCCGCTGCCACTACCGGCGTAGCGCTGGCGGAG		(GSGSG)
21	TCAGGGTGCCGAGCTCGTCCCCGTG	<i>His</i> ₆ -TEV- <i>Tt</i> TF	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{PPD}
22	AGCTCGGCACCCTGAAAATACAGGTT		(step 1)
23	CCCCGAGTGACTGGAAGTTCTGTTC	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{PPD}	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{PPD}
24	TCCAGTCACTCGGGGAGCTTCAGGGT	(step 1)	
17	CTACGCCGAGCTGGACGAGGAGTTC	<i>His</i> ₆ -TEV- <i>Tt</i> TF	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{RBD-SBD}
18	TCCAGCTCGGCGTAGCGCTGGCGGAG		
19	GGTAGTGGCAGCGGAGAGCTGGACGAGGAGTTC	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{RBD-SBD}	<i>His</i> ₆ -TEV- <i>Tt</i> TF ^{RBD-SBD}
20	TCCGCTGCCACTACCGGCGTAGCGCTGGCGGAG		(GSGSG)

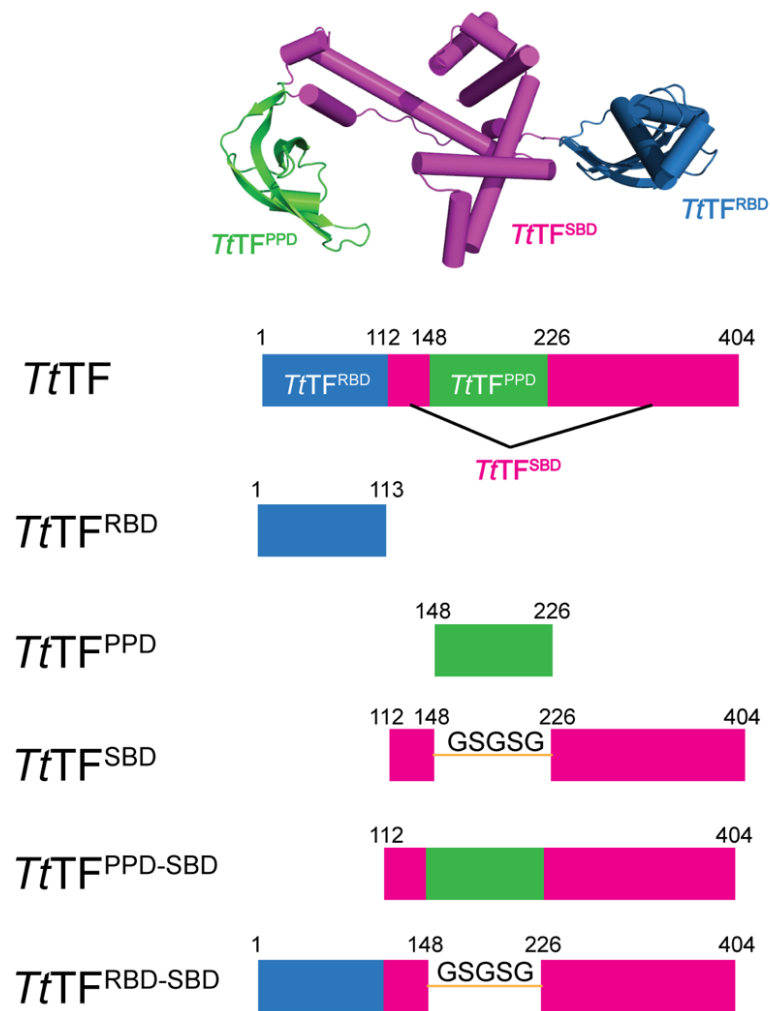


Figure S1. The domain architecture of *TtTF*. The domain architecture of *TtTF* and the constructs used in MALDI-TOF-MS experiments are shown with predicted structure of *TtTF*. The coordinate of *TtTF* was calculated by AlphaFold2 (<https://colab.research.google.com/drive/1LVPSOf4L502F21RWBmYJJYYLDIOU2NTL>, Accessed date: 17 August 2021).

EcTF MQV.SVE TTQGLGRRVTITIAADSIETAVKSELVNVAKKVRIDGFRKGKVP MNIVAQRYG
TtTF MAE.ILEERS.GYLVKVRVEVPADRVKASYEAL LKDLASRVRVPGFRPGKAPLKVV EARLG
TmTF MEVKELEERD.KNRVVLEYVFGAEETIAQAEDKAVRYLNQ RVEIPGFRKGRI PKNV LKMLKG

EcTF AS.VRQDVLGDLMSRNFIDAI..IKEKINPAGAPTYVPG EYKLGEDFTYSVEFEVYPEVE
TtTF REALLQDLKERLVEETYP EAV..RELGLSPVAAR.VVEQDLSEGEGRFYVAEVENYPEVVR
TmTF EE.FQEYTLDFLMDLIPDTLKDRKLI LSPI....VTERELKDVT.ARVVVEVHEEPEVVR

EcTF LQGLEAIEVEKPI.VEVT DADVDGMLDTLRKQQA TWKEKDGAVEAEDRVTIDFT.GSV DGG
TtTF LPDWRSFALVESP.PEVTEEMVEKALEELRQRYAELVPV EREAQEKDHLFVR....TEEG
TmTF IGDISKIEVEKVDEEKVLEKYVERRIEDLRESHALLEPKEGPAEAGDLVRVNMEVYN EEG

EcTF EEFEGGKASDFVLA MGQGRMI PGFEDG IKGHKAG EEF TIDVTFPEEYHAENLKGK.AAKF
TtTFAEF..PIDLAKALPHVR EALLGKKAGDVVMVPVL.....NDKGEKVREV
TmTF KKLT.SREY EY..VISEDEDRPFVKD.LVGKKKG DVVEI ERE.....YEGK.KYTY

EcTF AINLKKVEERE LPELTAEFIKRFGVEDGSVEGLRAEVRKNMERELKSAIRNRVKSQAIEG
TtTF RTEVLEVKTLKLPELDEEF AKTL..EAE SLEDLKNRVR ESKRQAERAYEEARERAFLEK
TmTF KLEVEEVYKRTLPEIGDELAKSVNN EFE TLEQLKES LKKEGKEIYDVEMKESMREQLEK

EcTF LVKAN DIDVPAALIDSEIDVLR.....RQAA....QRFGGNEKQAL ELPRELFE EQA
TtTF LAEGL EVEIPPSMLRAEERHLL EHLAEDLYRQGISLEAYLEALKEKGEL EKFQEDLRKEA
TmTF LPEIV EIEI SDRTL EILVNEI....NRLK REGR....YEQIVSSYSE EKFRELKERI

EcTF KRRVVVGLLLGEVIRTNELKAD EERVKGLIEEMASAYEDPK.EVIEFY SKNKELMDNMNRN
TtTF EKRVRIALAREKLAEELNPEVSEEEWQAYLQAAARAYGVAV.....QDLRRQFGE
TmTF LDDIKRDRVIEVLAQEKGISVND EEELEKEAEELAPFWGISPDRAKSLVKARQDLREELRW

EcTF VAL EEQAVEAVLAKAKVTEK.ETTFNELMNQQA....
TtTF EGLAR.LKERLRQDKAVQEALKALG.....
TmTF AILKRKVLDLL LQEVKV KVV.EPKG.EGDDSEGKEDN

Figure S2. Amino acid sequence alignment of *EcTF*, *TtTF*, and *TmTF*. The conserved amino acid residues are shown in blue frame and the identical residues are highlighted in red background. The identity and similarity between *EcTF* and *TtTF* are 26% and 49%, respectively. The identity and similarity between *TtTF* and *TmTF* are 35% and 52%, respectively. The identity and similarity between *EcTF* and *TmTF* are 24% and 45%, respectively. The sequence alignment was performed operated on the BLAST web server (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, Accessed date:14 April 2021, and 9 October 2021).

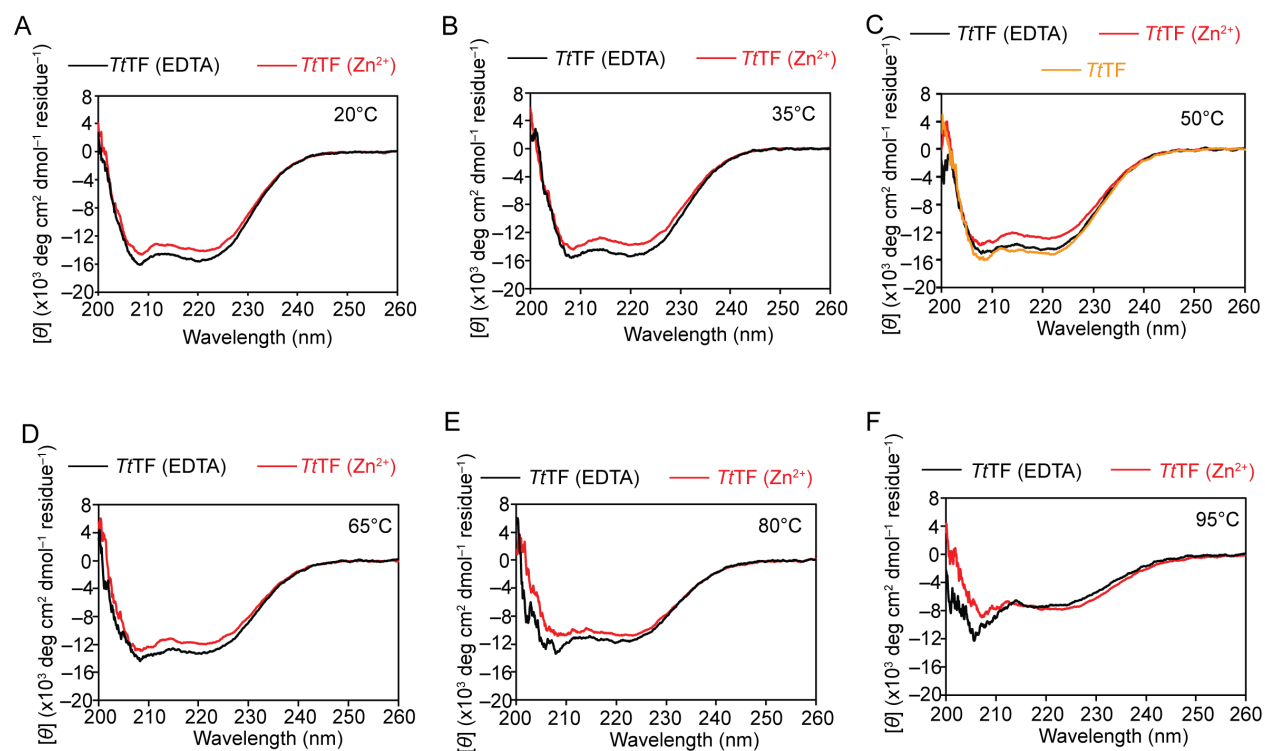


Figure S3. The CD spectra of *TtTF* at varying temperatures. The CD spectra of *TtTF* (Zn^{2+}) (red) and *TtTF* (EDTA) (black) at 20°C (A), 35°C (B), 65°C (C), 80°C (D), and 95°C (E) from 200 nm to 260 nm are displayed. The CD spectrum of *TtTF* before refolding at 50°C (orange) (C) is superimposed, showing that the spectrum of *TtTF* before refolding is essentially the same as that of *TtTF* (EDTA).

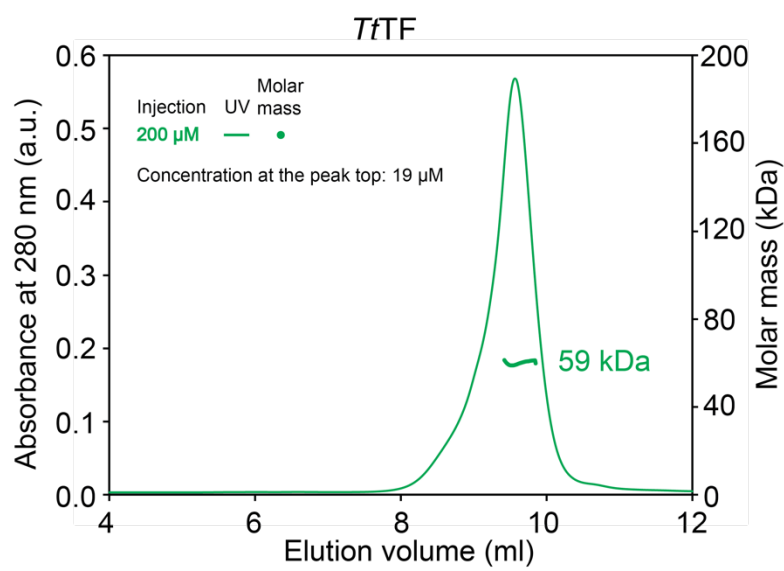


Figure S4. SEC-MALS profile of the natively purified *Tt*TF before refolding. The mass plots show that *Tt*TF exists mostly as a monomer at $\sim 19 \mu\text{M}$, which coincides with the SEC-MALS data of *Tt*TF (EDTA).