STRUCTURAL BIOLOGY
COMMUNICATIONS

Supporting information for article:

Crystal structure of thermally stable homodimeric cytochrome *c′*-β from *Thermus thermophilus*

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Signal peptide sequence

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EcoRI     10          20          30          40          50          60
          M   K   P   Y   A   L   L   S   L   L   A   T   G   T   L   L   A   Q
      GAATTCATGA AACCGTATGC ACTGCTGAGC CTGCTGGCAA CCGGTACACT GCTGGCACAA
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Mature protein sequence

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          70          80          90         100         110         120
      G   A   W   A   G   L   P   Y   P   E   G   Y   R   F   W   T   H   V   K   S
      GGTGCATGGG CAGGTCTGCC GTATCCGGAA GGTTATCGTT TTTGGACCCA TGTTAAAAGC

         130         140         150         160         170         180
      M   E   L   K   P   G   H   P   L   Y   E   S   F   G   L   H   H   I   Y
      ATGGAACTGA AACCGGGTCA TCCGCTGTAT GAAAGCTTTG GTGGTCTGCA TCATATTTAT

         190         200         210         220         230         240
      V   N   P   T   G   L   R   T   Y   L   E   G   K   K   A   P   F   P   K   G
      GTTAATCCGA CCGGTCTGCG TACCTATCTG GAAGGTAAAA AAGCACCGTT TCCGAAAGGC

         250         260         270         280         290         300
      T   V   I   V   F   D   L   L   E   A   K   V   E   G   N   A   L   L   E   G
      ACCGTTATTG TTTTTGATCT GCTGGAAGCA AAAGTTGAAG GTAATGCACT GTTAGAAGGT

         310         320         330         340         350         360
      P   R   K   L   I   G   V   M   A   K   D   P   G   R   Y   P   D   T   G   G
      CCGCGTAAAC TGATTGGTGT TATGGCAAAA GATCCGGGTC GTTATCCTGA TACCGGTGGT

         370         380         390         400         410         420
      W   G   Y   Y   A   F   G   P   D   K   K   P   L   A   I   D   P   K   A   C
      TGGGGTTATT ATGCATTTGG TCCGGATAAA AAACCGCTGG CAATTGATCC GAAAGCATGT

         430         440         450         460         470         480
      H   A   C   H   Q   G   A   A   N   T   D   Y   V   F   S   A   F   R   P   *
      CATGCCTGTC ATCAGGGTGC AGCAAATACC GATTATGTTT TTAGCGCATT TCGTCCGTAA

         490         500         510         520         530         540
Sall

GTCGAC
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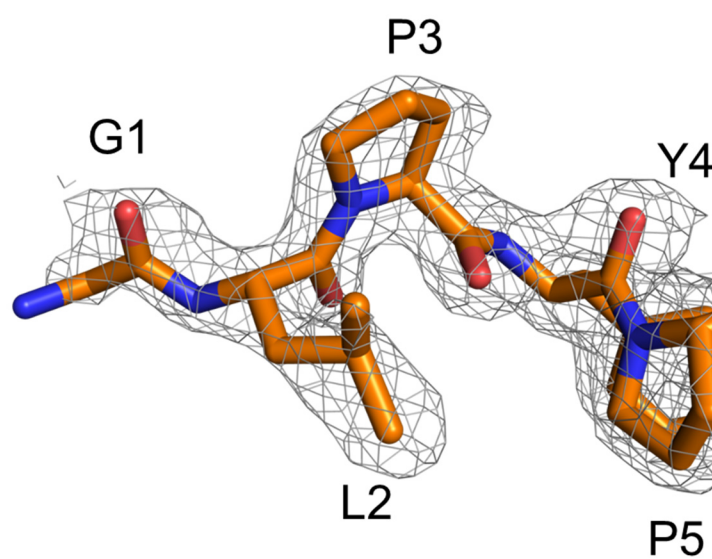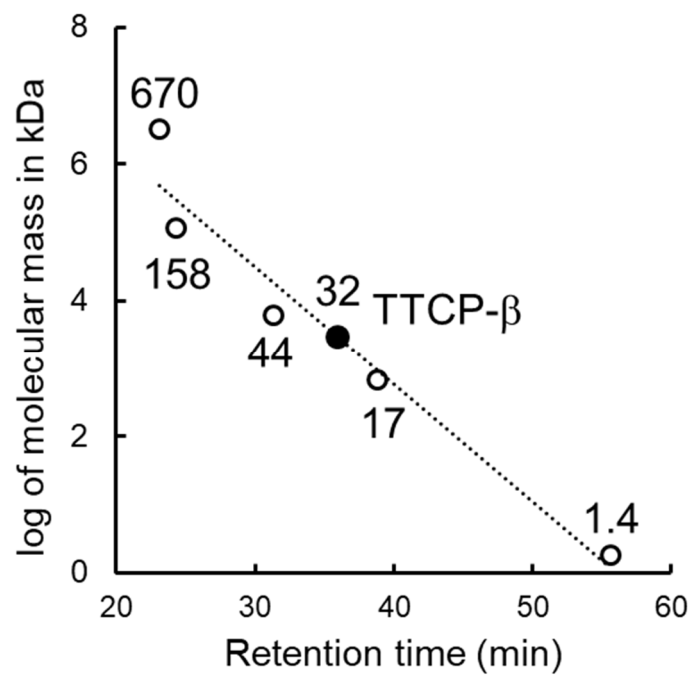
Figure S1 Synthesized gene for TTCP-β protein expression.

Figure S2 $2F_o$-DF_c electron density map countered at 1.0 σ showing the N-terminal region of the

TTCP-β protein.

Figure S3 Molecular mass determination by gel filtration chromatography. The logarithm of

marker molecular mass is plotted (open circles). The marker molecular mass values are also

indicated in kDa for each point. The retention time of TTCP-β is plotted on the linear least-square

fitting curve (closed circle).

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Methyloglobulus morosus        1 ---------QKDGVPFPEGYRNWLHAKSMLIQPGHALENPFQGIHHVYANGKAAKGLKS 50
Methylomicrobium buryatense    1 ---------AEQAVPFPEGYRNWLHTKSMLIQPGHALEDPFQGIHHIYANKLAEDGLKT 50
Methylohalobius crimeensis     1 ---------GTSVEYPEGFRTWYHVKSMVIQPGHPLEDPFGGIHHIYANYKALQGLKS 49
Methylocmonas koyamae          1 ---------AEHSVAYPEGYRTWLHAKSMLIQPGHALENPFQGLHHVYANKKAESGLKS 50
Pseudomonas guangdongensis     1 ---------AEPEVAYPEGYRDWRHVKSMLIEEGHPLHAAFGGIHHIYANDTALEGYRN 50
Pseudomonas linyingensis       1 ---------ADPEVPYPSGYRHWQHVKSMVIEQGHPLFDAFGGIHHIYANASALEGYQA 50
Pseudomonas sagittaria         1 ---------ADPEVPYPTGYRHWHHVKSMVIEQGHPLFEAFGGIHHLYANAAALEGYQA 50
Geobacter bemidjiensis         1 ----------AGQTALPKGYEKWDKSKARIDTDKKSL---FYGIHYIYVDKKAMKSYKS 46
Geobacter daltonii             1 ----------ATGTLPKGYEKWEKSKEKVVSDKSSL---FYGVHNIYVDKKAMPAYKA 45
Geobacter pickeringii          1 ----------ADTATLPKDYEKWAKSKEKVISDKSSL---FYGIHHIYVDKKAMPPYQS 46
Geobacter pelophilus           1 ----------AQTALPRGYEKWEKSKARINTDKKSL---FYGIHYIYVDKKAMKGYKS 45
Geobacter uraniireducens       1 ----------AEKASLPKGYEKWEKSKQKIVNDKKSL---FYGIHYTYVDKKALKAFKT 46
Geobacter lovleyi              1 ---------ATSTPPLPK-YQGWTKSVRKVVTDKKSL---FYGIHYIYADPKAIKGYQA 46
Geobacter sulfurreducens       1 ----------AGASLPKGYEKWNMSKEKVIADKSSL---FYGIHNIYVDKKAMAAYRK 45
Methylococcus capsulatus       1 ---------AETKVKYPDGFRSWYHVKSMVIQPGHPLENPFGGIHHVYANAEAIQGLRG 50
Ignavibacterium album          1 ---AVLFGFSNDEVKYPEGYRNWTHVKTLILEKGHPLYDAFGGIHHIYANTTALEGYKA 56
Fontimonas thermophila         1 ---------GADQVPYPQGYRNWVHVKSMVIQEGHPLFASFGGIHHIYGNAKAIAGYEA 50
Meiothermus rufus              1 ---------------PEGYRFWTHIKSMEIGPGHPLYEAFGGLHHIYANPQALAGYLS 43
Meiothermus ruber              1 -----VSGQTVEGVAFPEGYRTWTHVKSMIVEKGHPLFELVGGLHHIYANSKAMQGYQA 54
Thermoanaerobaculum aquaticum  1 ------LAEKAKGVSYPEGYRRWYHVKTLVLQAGHPLYDAFGGIHHVYANEKAKQGLST 53
Thermus amyloliquefaciens      1 -AQYGGG-VSQPAFPYPEGYRLWTHVKSMELKPGHPLYESFGGLHHIYVNQTGLRTYLE 57
Thermus tengchongensis         1 QYQYGSDQASPPTFPYPEGYRLWTHVKSMELKPGHPLYESFGGLHHIYVNQTGLKTYLE 59
Thermus arciformis             1 ----------ETGFPYPEGYRLWTHVKSMELKPGHPLYESFGGLHHVYNPVGLKTYLE 49
Thermus oshimai                1 ----------QEGFPYPEGFRFWTHVKSMEIQRGHPLYEAFGGLHHIYANPQALAGYLS 49
Thermus thermophilus           1 ------------GLPYPEGYRFWTHVKSMELKPGHPLYESFGGLHHIYVNPTGLRTYLE 47
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Methyloglobulus morosus        51 G---KYDDGSVLVFDLLKYEEKDKTIQEGERKLVGVMRKDSTKYASTGGWGFEGFTGNS 106
Methylomicrobium buryatense    51 G---KYKDGAVLVFDLLNYVEKDKTIEEGDRKLIGVMLKDAKKFADTGGWGFEGFAGDS 106
Methylohalobius crimeensis     50 G---DYPDGAVLVFDLLDYQSSNNALVEGKRKLIGVMERDAERFAATGGWGFEGFGGGD 105
Methylocmonas koyamae          51 G---KYEDGSVLVFDLLQYQEKDKTIQEGERKLVGVMQKDSKKYAATGGWGFEGFAGNS 106
Pseudomonas guangdongensis     51 G---RFADGAVIVFDLLEAKRSDAAVVEGARKVVGVMHKDSQRFAATGGWGFEGFAGGD 106
Pseudomonas linyingensis       51 G---KFPDGAVIVFDLLEANRADSAVTEGARKVVGVMHKDAQKYAATGGWGFEGFAGGD 106
Pseudomonas sagittaria         51 G---KFPDGAVIVFDLLEAQRADSAVTEGARKVVGVMHKDAQKYAATGGWGFEGFAGGD 106
Geobacter bemidjiensis         47 G--GAYPDGSRFVAVNYSIKEENGNKVPGKKNMIVVMQKD-RKEQQTGGWRFAGFTPDG 102
Geobacter daltonii             46 G--GKYPEGSRFVVVDYYKLKEENGKPVKGKKNMIVLMKKD-KKFKETGGWQFVGFDKF 101
Geobacter pickeringii          47 G--GKYSEGARFVVVNYTIKEEGGKPVKGKKSMVVLMKKD-KKQSATGGWLFAGFTPEG 102
Geobacter pelophilus           46 G--GAYPDGSRFVAVNYSIKEENGKQVPGKKNMIVVMQKD-RTQQQTGGWRFAGFTPEG 101
Geobacter uraniireducens       47 G--GKYPEGSRFVVDYYNIREEGGKPVQGKKNMIVLMKKD-KKFKETGGWQFAGFNPDG 102
Geobacter lovleyi              47 G--NKFPEGSRIVVEHFNIKGDNS-SIDGPKNMVVLMKKD-KTRKATGGWLYAGYTAEG 101
Geobacter sulfurreducens       46 G--GPYPEGSRFVVVQHTIKDVGGKPTKGKRSMIVLMTKD-KKQTATGGWLFAGFTAEG 101
Methylococcus capsulatus       51 G---NYPDGAVLVFDLFDYQEDNHALVEGKRKLIGVMERDAKRFSATGGWGYEGFGEGK 106
Ignavibacterium album          57 G--NKFKDGSVIVFDLLETVSADNAVAEGNRKVVGVMEKNSKKYRDTGGWGFEAFKG-D 112
Fontimonas thermophila         51 G---RFEDGAVIVFDLLEAQADGHALVEGPRKVLGVMHKDARRYAATGGWGFEGFAGGD 106
Meiothermus rufus              44 DRR-SFPDGSVIAFDLLEVRKEQGATLEGPRKLVGVMVKNSSRYARTGGWGFLAF-GPN 100
Meiothermus ruber              55 NPR-VFPEGAVIVFDLLEPVKAENAVVEGKRKAVIVMEKDTRRFAATGNWGYAVFEGDS 112
Thermoanaerobaculum aquaticum  54 G---KYANGAVFVFDLLEAPVEGNALVEGPRKVLAVMEKDSNRFADTGGWGPQAFSAGD 109
Thermus amyloliquefaciens      58 GKKNPFFPKGTVIVFDLLEAKVEGNALLEGPQKLIGVMVKDPDRYPATGGWGYYAF-GPD 115
Thermus tengchongensis         60 GKKDPFFPKGTVIVFDLLEAKAEGNALLEGPRKLIGVMVKDPDRYRDTGGWGYYAF-GPD 117
Thermus arciformis             50 GKKAPFPRRTVIVFDLLEAKVEGNALLEGPRKLIGVMVKDPERYRETGGWGYYAF-GPD 107
Thermus oshimai                50 GKR-AFPEGSVIVFDLLEARAEGQALVEGPRKLIGVMVKDSRRYPETGGWGYYAF-GAD 106
Thermus thermophilus           48 GKKAPFPKGTVIVFDLLEAKVEGNALLEGPRKLIGVMAKDPGRYPDTGGWGYYAF-GPD 105
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			Pro number	Optimal bacterial growth (°C)	
Methyloglobulus morosus	107	KTERLV-NDGGKSCFGCH-EPQKGKNYVFSGLRD-- 138	5	20	(Deutzmann et al., 2014)
Methylomicrobium buryatense	107	KTERLV-TDGGQSCFGCH-APQKKTDYVFSEFRK-- 138	5	28-30	(Kaluzhnaya et al., 2001)
Methylohalobius crimeensis	106	PSKRLV-TDGGQGCYGCH-TSQKDSQFVFSKLRD-- 137	6	30	(Heyer et al., 2005)
Methylocmonas koyamae	107	KTERLV-KDGGASCFACH-AAEQKTDYVFSQYRP-- 138	4	30	(Ogiso et al., 2012)
Pseudomonas guangdongensis	107	RQRRAVAGDAATACFACH-EPEREHDYVFSRARD-- 139	4	30	(Yang et al., 2013)
Pseudomonas linyingensis	107	PSKRAVGANAATACYACH-APEKDRDYVFSRPRD-- 139	8	30	(He et al., 2012)
Pseudomonas sagittaria	107	PTKRTVGADAATACYACH-APEKDHDYVFSRPRD-- 139	8	30	(Lin et al., 2013)
Geobacter bemidjiensis	103	KPS-GL--DGKRDCFGCHEKDAKGRDYVISYADFK 135	5	30	(Nevin et al., 2005)
Geobacter daltonii	102	KAS-KL--DPVKNCYECHLEAKDRDYVISKYTDFK 134	5	30	(Prakash et al., 2010)
Geobacter pickeringii	103	KPS-GI--DPVKNCFECHLDAKATDLIISKYADFK 135	7	30	(Shelobolina et al., 2007)
Geobacter pelophilus	102	KPS-GL--DGKRDCFGCHEKDAKDRDYVISRYTDFK 134	5	30-32	(Straub et al., 2001)
Geobacter uraniireducens	103	RPS-AV--DPVKNCYECHLEAKDADLVISKYADFK 135	6	32	(Shelobolina et al., 2008)
Geobacter lovleyi	102	KPS-RL--DPVKNCFECHQKEVAGRDYIFSGIADFK 134	8	35	(Sung et al., 2006)
Geobacter sulfurreducens	102	KPS-GV--DTVKNCYECHLKEAGSRDLVISRYADFK 134	5	35	(Caccavo et al., 1994)
Methylococcus capsulatus	107	PDKRLV--TDGGQGCFGCHAAQKESQYVFSRLRD-- 138	6	45	(Lund et al., 1985)
Ignavibacterium album	113	TRERVV-KNMEGDCFSCH-LSQKDKDYVFSEYRK-- 144	2	45	(Iino et al., 2010)
Fontimonas thermophila	107	PKQRVVGENAKTACFDCH-ASQAGHDYVFSRKRDR- 140	5	45-50	(Losey et al., 2013a)
Meiothermus rufus	101	KAPLSI---DAQACFTCH-QGAASSDYVFSTYRP-- 130	9	55-60	(Albuquerque et al., 2009)
Meiothermus ruber	113	RRPVEI---NANSCYECH-RGAANTDFVFSSPRP-- 142	7	60	(Ferreira et al., 1999)
Thermoanaerobaculum aquaticum	110	PQKPIV-VNAKEQCFSCH-ESQKDKDFVFSTWRP-- 141	6	60	(Losey et al., 2013b)
Thermus amyloliquefaciens	116	KKPLSI---DPQSCHACH-QGAANTDFVFSAFRP-- 145	14	60-65	(Ming et al., 2020)
Thermus tengchongensis	118	KRPLSI---DPQSCHACH-QGAANTDFVFSAFRP-- 147	14	65	(Yu et al., 2013)
Thermus arciformis	108	KKPLAI---DPKACHACH-QGAANTDYVFSAFQP-- 137	13	70	(Zhang et al., 2010)
Thermus oshimai	107	GKPLAI---DPKACHACH-QGAANTDFVFSAFRP-- 136	10	70	(Williams et al., 1996)
Thermus thermophilus	106	KKPLAI---DPKACHACH-QGAANTDYVFSAFRP-- 135	14	72	(Oshima et al., 1974)

Figure S4 Amino acid sequence alignment of 25 cytochromes c'-β. The red box corresponds to the amino acids mentioned in the main text. The bars indicate the sequence gaps. Number of Pro residues and optimal growth temperatures of source bacteria are also shown.

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