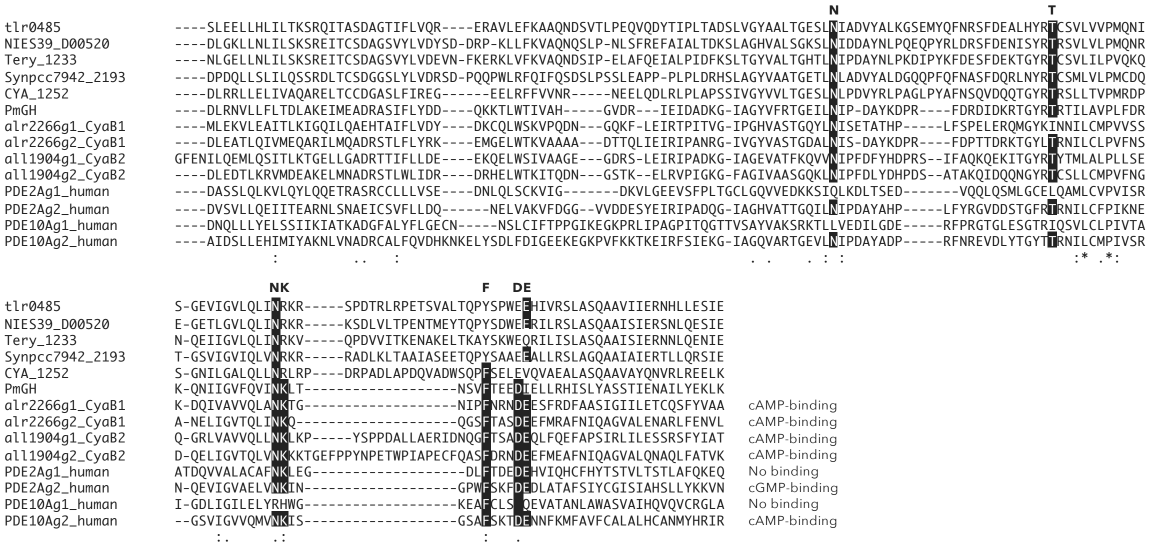
Supplementary Figures



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                                                                                                  N                          T
tlr0485          ----SLEELLHLILTKSRQITASDAGTIFLVQR----ERAVLEFKAAQNDSVTLPEQVQDYTIPLTADSLVGYAALTGESLNIADVYALKGSEMYQFNRSFDEALHYRTCSVLVVPMQNI
NIES39_D00520    ----DLGKLLNLILSKSREITCSDAGSVYLVDYSD-DRP-KLLFKVAQNQSLP-NLSFREFAIALTDKSLAGHVALSGKSLNIDDAYNLPQEQPYRLDRSFDENISYRTRSVLVLPMQNR
Tery_1233        ----NLGELLNLILSKSREITCSDAGSVYLVDEVN-FKERKLVFKVAQNDSIP-ELAFQEIALPIDFKSLTGYVALTGHTLNIPDAYNLPKDIPYKFDESFDEKTGYRTCSVLILPVQKQ
Synpcc7942_2193  ----DPDQLLSLILQSSRDLTCSDGGSLYLVDRSD-PQQPWLRFQIFQSDSLPSSLEAPP-PLPLDRHSLAGYVAATGETLNLADVYALDGQQPFQFNASFDQRLNYRTCSMLVLPMCDQ
CYA_1252         ----DLRRLLELIVAQARELTCCDGASLFIREG------EELRFFVVNR-----NEELQDLRLPLAPSSIVGYVVLTGESLNLPDVYRLPAGLPYAFNSQVDQQTGYRTRSLLTVPMRDP
PmGH             ----DLRNVLLFLTDLAKEIMEADRASIFLYDD----QKKTLWTIVAH-----GVDR---IEIDADKG-IAGYVFRTGEILNIP-DAYKDPR----FDRDIDKRTGYRTRTILAVPLFDR
alr2266g1_CyaB1  ----MLEKVLEAITLKIGQILQAEHTAIFLVDY----DKCQLWSKVPQDN---GQKF-LEIRTPITVG-IPGHVASTGQYLNISETATHP-----LFSPELERQMGYKINNILCMPVVSS
alr2266g2_CyaB1  ----DLEATLQIVMEQARILMQADRSTLFLYRK----EMGELWTKVAAAA---DTTQLIEIRIPANRG-IVGYVASTGDALNIS-DAYKDPR----FDPTTDRKTGYLTRNILCLPVFNS
all1904g1_CyaB2  GFENILQEMLQSITLKTGELLGADRTTIFLLDE----EKQELWSIVAAGE---GDRS-LEIRIPADKG-IAGEVATFKQVVNIPFDFYHDPRS--IFAQKQEKITGYRTYTMLALPLLSE
all1904g2_CyaB2  ----DLEDTLKRVMDEAKELMNADRSTLWLIDR----DRHELWTKITQDN---GSTK--ELRVPIGKG-FAGIVAASGQKLNIPFDLYDHPDS--ATAKQIDQQNGYRTCSLLCMPVFNG
PDE2Ag1_human    ----DASSLQLKVLQYLQQETRASRCCLLLVSE----DNLQLSCKVIG-------DKVLGEEVSFPLTGCLGQVVEDKKSIQLKDLTSED-------VQQLQSMLGCELQAMLCVPVISR
PDE2Ag2_human    ----DVSVLLQEIITEARNLSNAEICSVFLLDQ------NELVAKVFDGG--VVDDESYEIRIPADQG-IAGHVATTGQILNIPDAYAHP-----LFYRGVDDSTGFRTRNILCFPIKNE
PDE10Ag1_human   ----DNQLLLYELSSIIKIATKADGFALYFLGECN-----NSLCIFTPPGIKEGKPRLIPAGPITQGTTVSAYVAKSRKTLLVEDILGDE-----RFPRGTGLESGTRIQSVLCLPIVTA
PDE10Ag2_human   ----AIDSLLEHIMIYAKNLVNADRCALFQVDHKNKELYSDLFDIGEEKEGKPVFKKTKEIRFSIEKG-IAGQVARTGEVLNIPDAYADP-----RFNREVDLYTGYTTRNILCMPIVSR
                             :         ..   :                                           . .    . : :                            :* .*:

                             NK                        F   DE
tlr0485          S-GEVIGVLQLINRKR-----SPDTRLRPETSVALTQPYSPWEEHIVRSLASQAAVIIERNHLLESIE
NIES39_D00520    E-GETLGVLQLINRKR-----KSDLVLTPENTMEYTQPYSDWEERILRSLASQAAISIERSNLQESIE
Tery_1233        N-QEIIGVLQLINRKV-----QPDVVITKENAKELTKAYSKWEQRILISLASQAAISIERNNLQENIE
Synpcc7942_2193  T-GSVIGVIQLVNRKR-----RADLKLTAAIASEETQPYSAAEEALLRSLAGQAAIAIERTLLQRSIE
CYA_1252         S-GNILGALQLLNRLRP----DRPADLAPDQVADWSQPFSELEVQVAEALASQAAVAYQNVRLREELK
PmGH             K-QNIIGVFQVINKLT-------------------NSVFTEEDIELLRHISLYASSTIENAILYEKLK
alr2266g1_CyaB1  K-DQIVAVVQLANKTG-------------------NIPFNRNDEESFRDFAASIGIILETCQSFYVAA  cAMP-binding
alr2266g2_CyaB1  A-NELIGVTQLINKQ--------------------QGSFTASDEEFMRAFNIQAGVALENARLFENVL  cAMP-binding
all1904g1_CyaB2  Q-GRLVAVVQLLNKLKP-----YSPPDALLAERIDNQGFTSADEQLFQEFAPSIRLILESSRSFYIAT  cAMP-binding
all1904g2_CyaB2  D-QELIGVTQLVNKKKTGEFPPYNPETWPIAPECFQASFDRNDEEFMEAFNIQAGVALQNAQLFATVK  cAMP-binding
PDE2Ag1_human    ATDQVVALACAFNKLEG-------------------DLFTDEDEHVIQHCFHYTSTVLTSTLAFQKEQ  No binding
PDE2Ag2_human    N-QEVIGVAELVNKIN-------------------GPWFSKFDEDLATAFSIYCGISIAHSLLYKKVN  cGMP-binding
PDE10Ag1_human   I-GDLIGILELYRHWG-------------------KEAFCLS QEVATANLAWASVAIHQVQVCRGLA  No binding
PDE10Ag2_human   --GSVIGVVQMVNKIS-------------------GSAFSKTDENNFKMFAVFCALALHCANMYHRIR  cAMP-binding
                      :.     .:                        :   .
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Fig. S1

The protein alignment of GAF domains of the Tlr0485 homologs and known cNMP-binding proteins. The amino acids crucial for cNMP-binding according to (Handa *et al.*, 2008) are highlighted in reverse color.

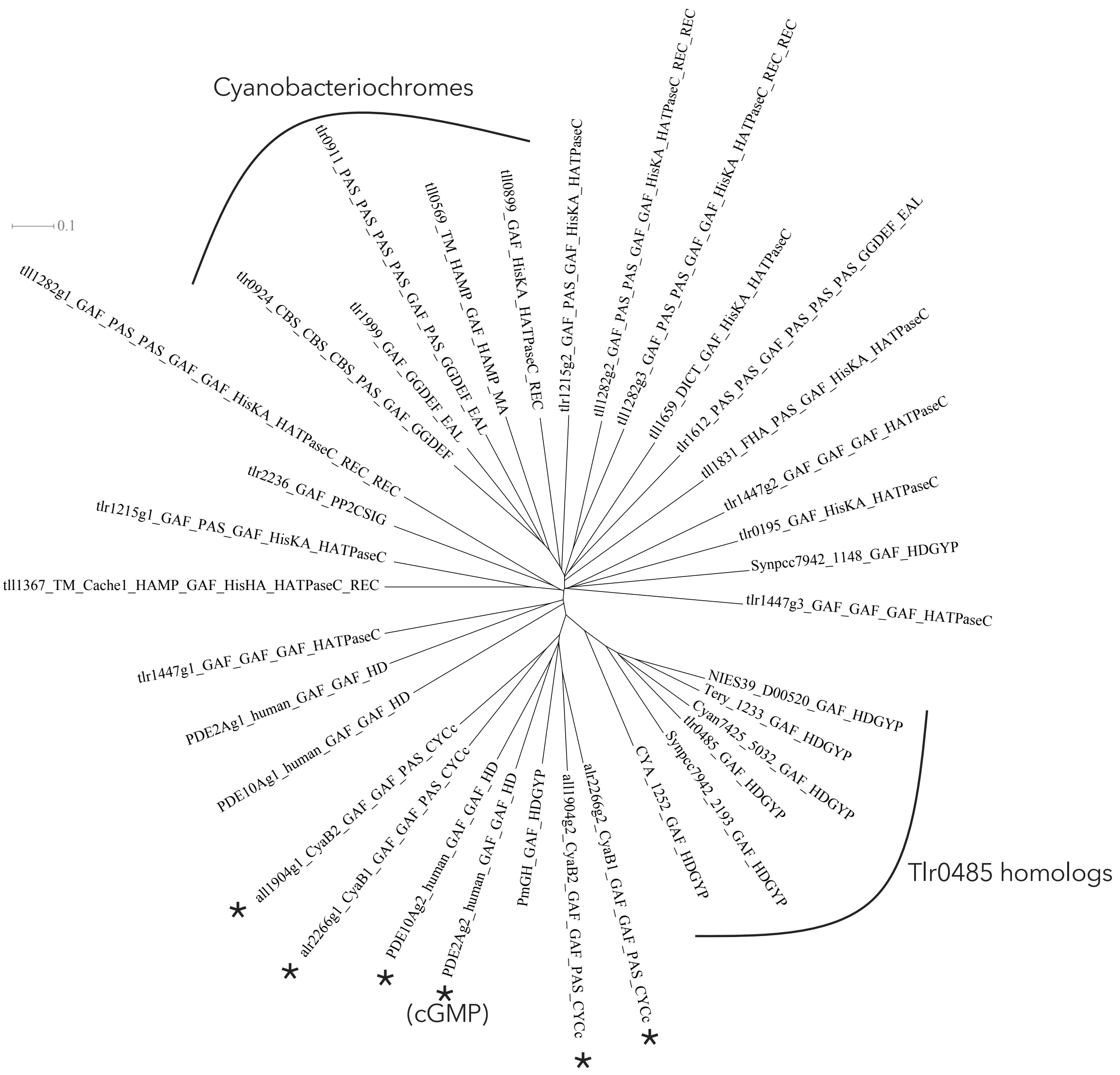

Fig. S2

The cluster analysis of GAF domains. The phylogenic trees for the GAF domains of Tlr0485 homologs, known cNMP-binding proteins, and all the GAF domain-containing proteins of *Thermosynechococcus elongatus* based on the neighbor-joining method. Sequence labels are protein names followed by the domain compositions of the full-length proteins. *: cAMP-binding GAF domains.

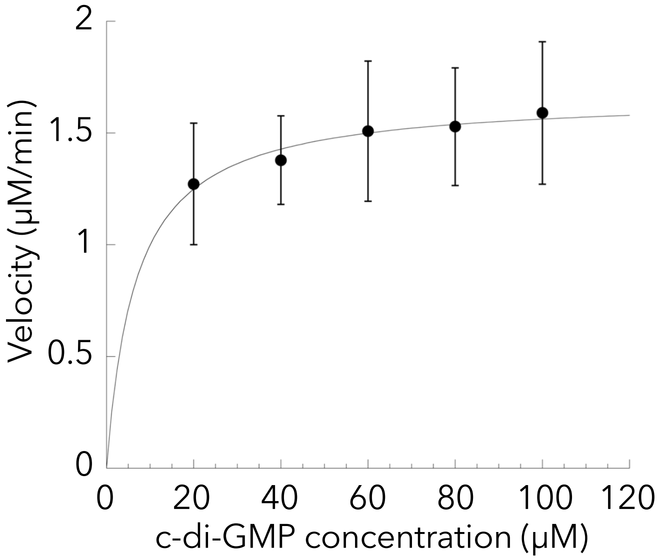

Fig. S3

The substrate concentration dependence curve for the PDE activity of Tlr0485 in the presence of 2 μM cAMP for activation of the enzyme. The reaction velocity is calculated from the amount of the reaction product, pGpG. Shown data are mean ± standard deviations of three independent experiments.

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tll2410_cya1        IALHNGDRITFGQTELDFFNEPFAPLFSENATLSLPQSEGSAT---ALLHVRRLLTVLVVDIRDFTQLTRQLE--EELLSELIGTWFHRAGEIIRQYGSWVDKYIGDAVMAVWIHESEEI
tll2280_cya2        ---------------------LLGQSISPEIAAALWERRDQLLKDGKLPGQRLIATLLFTDLKGFSTISERME--PEELFNWLNAYLEKVADVVQSYHGVINKFTGDGIMAVFGVPIKRT
Tvtll2410_cya1      IALHNGDRITFGQTELDFFNEPFAPLFAENATLSLPQSEGSAT---ALLHVRRLLTVLVVDIRDFTQLTRQLE--EELLSELIGTWFHRAGEIIRQYGSWVDKYIGDAVMAVWIHESEEI
Tvtll2280_cya2      ---------------------LLGQSISPEIAAALWERRDQLLKDGKLPGQRLIATLLFTDLKGFSTISERME--PEELFNWLNAYLEKVADVVQSYHGVINKFTGDGIMAVFGVPIKRT
slr1991_cya1        IAIQDQDKITFGKTEAQFYSDQNG---TPSGLLRNPAEWDTQT---NVLHERRLITVLVADMRNFTGMAQQVE--EELLSMLIGNWFRQAGHILREAGSWVDKYIGDAVMAIWFHGYNEA
sll0646_cya2        ----------------------FGRYLTDQVVATLLENPEGL----KMGGDRRPITILTSDLRGFTSTSEGLN--PEEVVKVLNIYFGKMADVITHHGGTIDEFMGDGILVLFGAPTSQQ
cyaA                -------------QIRDFVHNALGRYVSPDVAR--LVARDLS----LMRPERRKMSVYVCDIEGFTRLSESMP--PEQLVGLFNAYLTEISAVVRSTAGQVDKYIGDSVMAFWGAPVRTD
cyaB                -------------EEAAFIRQAFSRFMEPKLVEQMIAQRELP----RLDGENVEITAFFSDIRGFSTFSERFKDDPRNLVRLLNTYLTRVSAALLKEGGCLDKYIGDAVVCLFGAPMRQA
                                           .     .                 :   .   :    *:..*:  :. .    . :   :. :: . .  :    . :::: **.:: .:

tll2410_cya1        G----YQEICHTLSALEDLRLMTGELHQQYPLP--WPLRIGSGLNTGYAMVGNTGSGDRPDYTALGDTVNAAFRLESATKTIA---ADLAMGATTYHYLVHSCPTPVPFRP
tll2280_cya2        SREGIAIDARNAVDCALALGQLLEELNREWAAQGLPQVMMRAGIYTGPIVVGSLGSKNRLEYGVIGDSVNTASRLESVDKHRQPSPCRILVAQETLAYLGDRYEVEA----
Tvtll2410_cya1      G----YQEICHTLAALEDLRLMTGELHQQYPLP--WPLRIGSGLNTGYAMVGNTGSGDRPDYTALGDTVNAAFRLESATKTIA---ADLAMGATTYHYLVHSCPTPVPFRP
Tvtll2280_cya2      SREGIAIDARNAVDCALALGQLLEELNREWAAQGLPQVMMRAGIYTGPIVVGSLGSKNRLEYGVIGDSVNTASRLESVDKHRQPSPCRILVAQETLAYLGDRYEVEA----
slr1991_cya1        T----PAEIIQILHAVNRLQAMTAKLNQKYELP--FPLRIGTGINTGYAMVGNTGSGDHPDYTAIGDTVNAAFRLESATKQAH---FDLAMSEKTFSYLQDLPQWSATV--
sll0646_cya2        D------DALRAVACGVEMQLALREVNQQVTGLGLQPLEMGIGINTGEVVVGNIGSEKRTKYGVVGAQVNLTYRIESYTTGGQ-----IFISSTTLEAAGDRVHVNGNR--
cyaA                R------HAHLACEAALKMREVLTERQSAWEKKYGRRLSFRAAVDTGELVVGDLGSEMKSNYTVLGDAVGLASRLEAINKVYG---TYVLAGDTTAQLASGSYVFRV----
cyaB                D------HALRGCKGALAAKAAVDRLRDEFRAQGLPDMYTRIGVNTAVNFVGNFGSEQLFSYTAIGDGMNLAARLEGANKAYG---SVIMIGPRTYELAREHIEVRE----
                           .                . .          :    .: *.  .**. **    .* .:*  :. : *:*.  .        :  .  *
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Fig. S4

The protein alignment of type-III cyclase (CYCc) domains. cyaA and cyaB: adenylate cyclases of *Myxococcus xanthu*s. The amino acids crucial for ATP-binding are highlighted in black whereas those for GTP-binding are highlighted in grey (Ryu *et al.*, 2010).