

Supplementary materials

Polyamines disrupt the KaiABC oscillator by inducing protein denaturation

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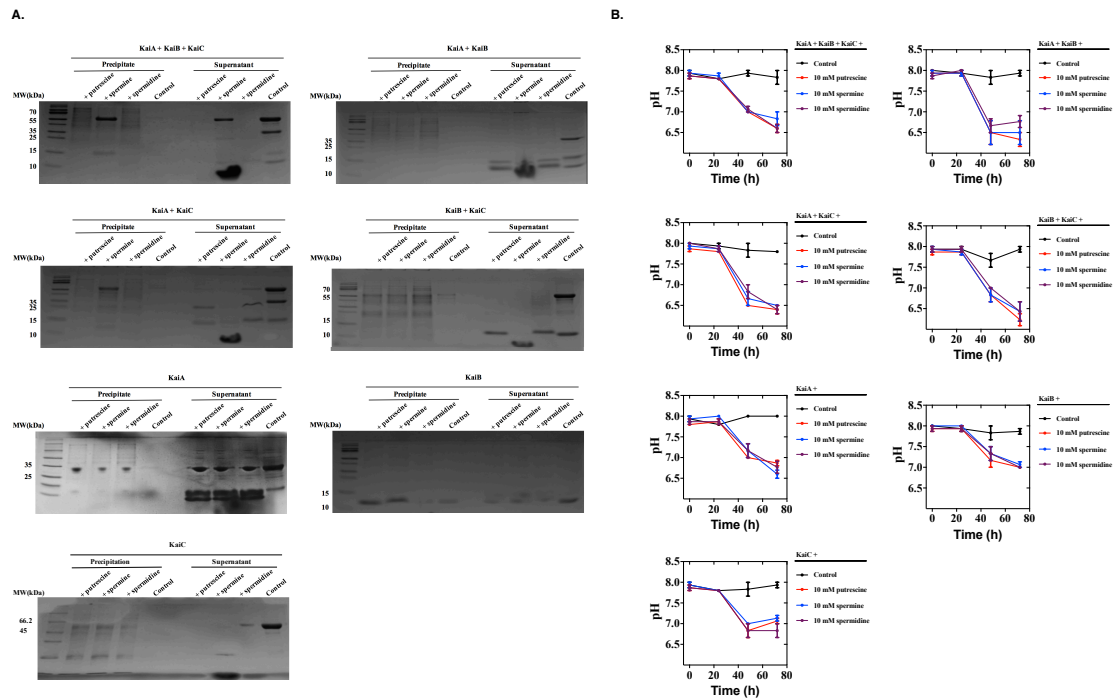


Figure S1. Polyamines caused the drop of solutions' pH values and protein denature after long-time incubation with Kai proteins. The samples without extra polyamines were controls. The total protein amounts of each lane were same. (A) The SDS-PAGE gels showed that the Kai proteins became less or invisible in the supernatants after being incubated for 72 hours with 10 mM of polyamines. Kai proteins became less or invisible in the supernatants. The additional protein bands at ~ 15 kDa were likely from the degradation of KaiA. (B) The pH values of the mixtures of Kai proteins and 10 mM polyamines dropped significantly after long-time incubation. The protein names are noted above the top lines.

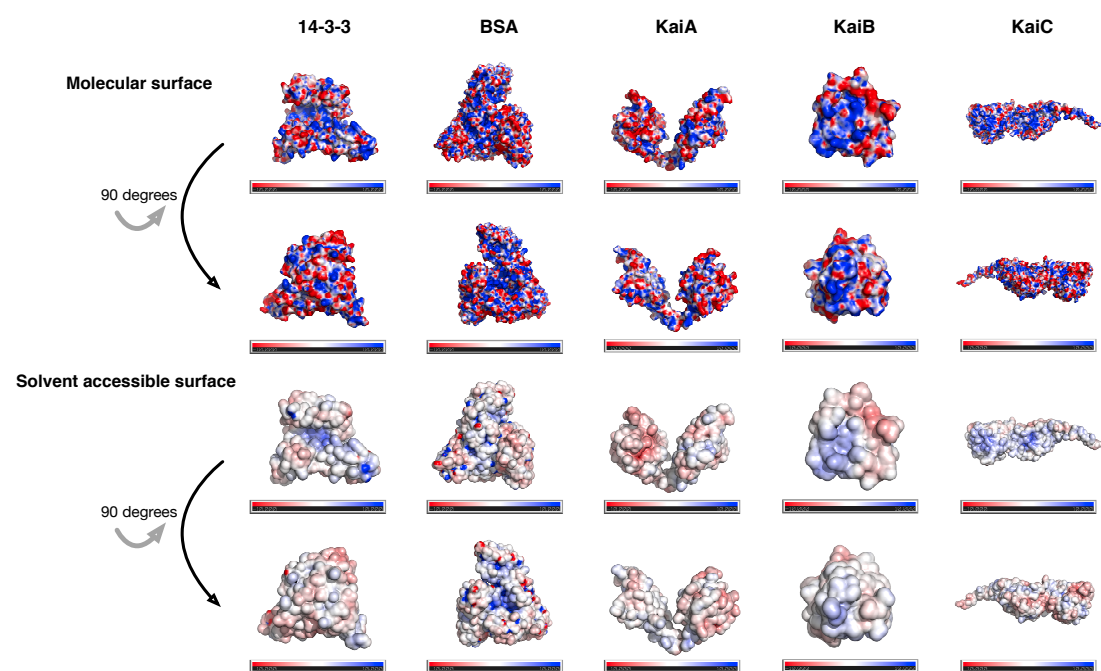


Figure S2. The electrostatic potentials of the protein surfaces were calculated with APBS (v2.1) in Pymol with the default settings. For each Kai protein, only a monomer in the homo-oligomers was shown. The Tau-K18 protein was not shown because its apo structure was not available. The PDB IDs were: 14-3-3, 3RDH; BSA, 3V03; KaiA, 5C5E; KaiB, 1R5P; KaiC, 2GBL.

Table S1. The sequences and cloning information of the proteins tested in this study.

Protein	Source	DNA coding sequence	Protein sequence	Restriction sites	Plasmid
KaiA	<i>Synechococcus</i> sp. PCC 7942	ctctcgcaaattgcaatctgcatttgggtggaatcgacggcaattttgcaggattgccagcggg cgctgtcggccgatcgctatcaactccaagtctgtgagcttgccgaaatgctcttgagtatgc ccaaacccatcgtgaccaaactgactgctgattttagtgagccaatcccagcttcagggc agttgttcagcagctctgctttgaggagtggtgtaccagcgattgtcgtaggcgatcgca cagttaggatcccgatgaaccagccaaagaacagctctatcacagcgctgaactgcacctc ggatccatcagctcgagcaattgccctaccaagtgtgctgactggctgaatttctgcgctt agccccggtcgagaccatggccgaccacatcatgctgagggggccaaccagatcccga gctatcgagccagcagcgggacctcgtcagcgactacaagagcgctaggctatctcggg gtctactacaagcgtgatcccgatcgctttctgcgcaacctacccgctacgaaagccaaaag ctgcaccaagcgatgcagactagctatcgtaaactggtttgagctattttcggcgaatgcaa cctcaaccagagcattgacaacttcgtcaacatggctttcttccgatgttccagtcaccaaag tggtagaaattcacatggagctgatggacgagtttgccaagaagctccgctagaggacgt tcagaggacatttctggtgattatcggtgactttaattgatgaattgcacatcttctgagatgt atcgacggctctatcccacgagaaacctga	GPLGSLSQIAICIWVESTAILQDCQ RALSadRYQLQVCESGEMLLEY AQTHRDQIDCLILVAANPSFRAV VQQLCFEGVVVPAIVVGDRDSED PDEPAKEQLYHSAELHLGIHQLE QLPYQVDAALAEFLRLAPVETM ADHIMLMGANHDPELSSQQRDL AQRLQERLGYLG VYYKRDPDRF LRNLPAYESQKLHQAMQTSYREI VLSYFSPNSNLNQSIDNFVNMAFF ADVPVTKVVEIHMELMDEFACK LRVEGRSEDILLDYRLTLIDVIAH LCEMYRRSIPRET	<i>Bam</i> H I <i>Not</i> I	pGEX-6P-1
KaiB	<i>Synechococcus</i> sp. PCC 7942	agccctcgtaaaacctacattctcaagctctacgtcgccggcaataactccaaactcagtcctgtg ccctcaaaacgctcaagaacattctcgaagtgaattcaaggtgttatgctctaaaggtgatc gatgttctcaaaaatcctcagttggcagaagaggataaaatcctagcgacgccaaacctcgc caaggttctaccactgcctgtccgacgattattggtgatttatccgacctgagaaaagtttga ttggccttgattactctacggcgaaactcaagattccgacgactctaa	GPLGSSPRKTYILKLYVAGNTPNS VRALKTLKNILEVEFQGVYALKV IDVLKNPQLAEEDKILATPTLAKV LPLPVRRRIIGDLSREKVLIGLDLL YGELQDSDDF	<i>Bam</i> H I <i>Not</i> I	pGEX-6P-1

KaiC	<i>Synechococcus sp.</i> PCC 7942	ggatccactccgctgagatgactagccctaataataattctgagcaccaagccatcgctaag atgcgcacgatgattgaaggctttgatgatattagtcatggcggtctccaatggcgcatoga ccctcgtagtggtacttcaggaaccggcaagaccctttttctattcaatttctctataacggatt atcgagtttgatgagcctgggggtttcgtacttcgaagaaaccccgaagatatcattaaaaa cgcccgtagttttggctgggatttagccaagctggcgcgatgagggcaactatttattcttgatg cttcaccgatccagaaggtaagaggtgttgccggcctcgcgcgatcctctgctctgattgagcg gattaattatgcaattcaaaagtatcgagcgcggcggttcaattgactcggtcacgtccgttt tcagcaatatgatgcctcttctgtgttcgccgcgaactctttcggttgtagctcgctaaaa caaattggggcaactacggcatgaccaccgagcgtatcgaggaatatggccgatcgcgc gtacgggtgttgaggaattgtctccgataacgtcgtgattctccgaacgttttggaaggga gcgccgtcgccgcacctcgaaatcctcaagctacgtggcaccagccacatgaaaggga atatccgttcacgattacggatcatggcatcaatatctccgctcggggcaatgcgccttacg cagcgatcgtcgaacgtgcgtgttcatctgtgtcgtccgactcgatgaaatgtgtgtggg ggctctttaaggactcaatcattctggcaactggcgcctacaggcactggtaaaactctgtagt tagccgtttcgttgagaatgctgtgctaacaagagcggcgcatctgttcgcttatgaagagt cacgagctcagctgctccgaatgcctattcatggggaatggactttgaggagatggagcgc caaaacctcctcaaaattgttgccctatcctgaatctgcaggtctgaagaccatttgagatt attaatcgagatcaatgactttaagccagctcgtattgcaatcgactccctctctgctttggc gcggggcggttagcaacaatgcctccgccaaattgtaattggtgtcactggctacgcgaaaca agaagaaatcacggactattcacaataaccagtgatcaatttatgggagcgcattcgattact gactcccatatctcaacaattacggatagcattatctgtccaatacgtcgagattcgtggcga aatgtcccgccgacgtcttcaagatgcgcggatcttgcatgacaaagcaatccgcga attcatgatcagcgacaaaggccggacatcaaggattcttccggaactttgagcggattatt tcaggttcgccaacacggattaccgtcgatgagaaaagcgaactctcgcaattgtgcgcgg cgttcaagaaaaaggccggagagctagcca	GPLGSGSTSAEMTSPNNNSEHQAI AKMRTMIEGFDDISHGGLPIGRST LVSGTSGTGKTLFSIQFLYNGIIEF DEPGVFVTFEETPQDIIKNARSFG WDLAKLVDEGKLFILDASPDPEG QEVVGGFDLSALIERINYAIQKYR ARRVSIDSVTSVFQQYDASSVVR RELFRLVARLKQIGATTVMITTERI EEYGPIARYGVEEFVSDNVVILRN VLEGERRRRTLEILKLRGTSHEMK GEYPFTITDHGINIFPLGAMRLTQ RSSNVRVSSGVVRLDEMCGGGFF KDSIILATGATGTGKTLLVSRFVE NACANKERAILFAYEESRAQLLR NAYSWGMDFEEMERQNLLKIVC AYPESAGLEDHLQIIKSEINDFKP ARIAIDSLSALARGVSNNAFRQFV IGVTGYAKQEEITGLFTNTSDQF MGAHSITDSHISTITDTIILLQYVEI RGEMSRainVFKMRGSHWDKAI REFMISDKGPDIKDSFRNFERIISG SPTRITVDEKSELSRIVRGVQKEG PES	<i>BamH</i> I <i>Not</i> I	pGEX-6P-1
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Tau-K18	Homo sapiens	atgggcagcagccatcaccatcatcaccacagccagatctggaacacgtattttcaggg atcccaaaccgcgccgtgccgatccggacctgaaaaacgttaagagcaaatcggtagc accgagaacctgaacaccagccgggtggcggtaagtgcataacataaagaactgg acctgagcaacgttcagagcaagtgcggcagcaagataacattaaacatgtccgggcgg tggcagcgtgcaaatgtttacaaccgggtggacctgagcaagtaccagcaagtgcggta gcctgggcaacatccaccacaagccgggtggcgggtcaggtgaggtgaagagcgaaaaac tggacttcaaagatcgtgtgcaagcaagatcggtagcctggataacattaccacgttccgg gcggtggcaacaagaaattgaataa	MGSSHHHHHHSQDLENLYFQGS QTAPVPMPLKLVKSKIGSTENL KHQPGGGKVQIINKKLDLSNVQS KCGSKDNIKHVPGGGSVQIVYKP VDLSKVTSK CGSLGNIHHKPGGGQVEVKS EKLDLFKDRVQ SKIGSLDNIT HVPGGGNKKIE	<i>BamH I</i> <i>EcoRI</i>	pET-28a
14-3-3	Homo sapiens	atgggcagcagccatcaccatcatcaccacagccagatctggaacacgtattttcaggg atccatggacaagaacgagctggtgcagaaggcgaaactggcggagcaagcgggaacgtta cgacgatatggcggcgtgcatgaagagcgtgaccgagcaggggtgcggaactgagcaacg aggaacgtaacctgctgagcgttgcgtataaaaacgtggttggtgcgcgtcgtagcagctgg cgtgtggttagcagcatcgaacaaaagaccgagggcgcggaagaaacagcaaatggc gcgtgagtaccgtgaaaaatcgagaccgaactgcgtgacatttgcaacgatgtgctgagcc tgctggagaagttcctgatcccgaacgcgagccagcggaagcaagtggttctacctgaa gatgaagggtgactactatcgttatctggcgggaagtggcggcgggtgacgataagaaaggc attgttgatcagagccagcaagcgtaccaagaggcgttcgaaatcagcaagaaagaaatgc aaccgacccaccgatctgtctgggtctggcgtgaacttcagcgtgttctactatgagattct gaacagcccggaagggcgtgcagcctggcgaaaaccgcgttgacgagggcgatcgagg aactggataccctgagcgaggaaagtataaagacagcacctgattatgcagctgctgcgt gataacctgacctgtggaccagcgacaccaaggtgatgaggcggaagcgggtgaggggt ggcgaaaactaa	MGSSHHHHHHSQDLENLYFQGS MDKNELVQKAKLAQAERYDD MAACMKSVTEQGAELSNEERNL LSVAYKNVVGARRSSWRVVSIE QKTEGAEKKQQMAREYREKIEIE LRDICNDVLSLLEKFLIPNASQAE SKVFYFLMKGDYYRYLAEVAAG DDKKGIVDQSQQAYQEAFAEISKK EMQPTHPIRLGLALNFSVFYYEIL NSPEKACSLAKTAFDEAIAELDTL SEESYKDSTLIMQLLRDNLTWT SDTQGDEAEAGEGGEN	<i>BamH I</i> <i>EcoRI</i>	pET-28a

BSA	Bovine	Not applicable (Commercial product)	DTHKSEIAHRFKDLGEEHFKGLV LIAFSQYLQQCPFDEHVKLVNEL TEFAKTCVADESHAGCEKSLHTL FGDELCKVASLRETYGDMADCC EKQEPERNECFLSHKDDSPDLPK LKPDPNTLCDEFKADEKKFWGK YLYEIAARRHPYFYAPELLYYANK YNGVFQECCQAEDKGACLLPKIE TMREKVLTSARQRLRCASIQKF GERALKAWSVARLSQKFPKAEF VEVTKLVTDLTKVHKECCHGDL LECADDRADLAKYICDNQDTISS KLKECCDKPLLEKSHCIAEVEKD AIPENLPPLTADFAEDKDVCKNY QEAKDAFLGSFLYEYSRRHPEYA VSVLLRLAKEYEATLEECCAADD PHACYSTVFDKCLKHLVDEPQNLI KQNCQFEKLGEGFQNALIVRY TRKVPQVSTPTLVEVSRSLGKVG TRCCTKPESERMPCTEDYLSLILN RLCVLHEKTPVSEKVTKCCTESL VNRPCFSALTPDETYVPKAFDE KLFTFHADICTLPDTEKQIKKQTA LVLLKHKPKATEEQLKTVMENF VAFVDKCCAADDKEACFAVEGP KLVVSTQTALA	Not applicable	Not applicable
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Table S2. The sequence compositions of the tested proteins could not explain the role of polyamines. (A) The sequence properties of the proteins studied in this work were compared. The parameters were calculated with ProtParam (<http://web.expasy.org/protparam/>). The sequences of the expressed proteins were used except BSA (Table S2). Glu and Asp were considered as negatively charged residues, whereas Arg and Lys were positively charged.

Name	Molecular weight (Da)	Theoretical pI	Positively charged residue ratio	Negatively charged residue ratio
KaiA	32912.52	4.83	8.68%	14.93%
KaiB	11716.69	6.47	13.21%	13.21%
KaiC	58427.31	5.81	11.81%	13.14%
BSA	66462.98	5.60	14.06%	16.98%
Tau-K18	16250.42	9.52	13.91%	8.61%
14-3-3	30312.80	4.93	11.61%	17.60%