Supplement

Design of a temperature-responsive transcription terminator

Johanna Roßmanith[1], Mareen Weskamp[1] and Franz Narberhaus[1,*]

[1] Microbial Biology, Ruhr University Bochum, 44780 Bochum, Germany

* To whom correspondence should be addressed. Tel: +49 (0)234 322 3100;
Fax: +49 (0)234 3214620; Email: franz.narberhaus@rub.de

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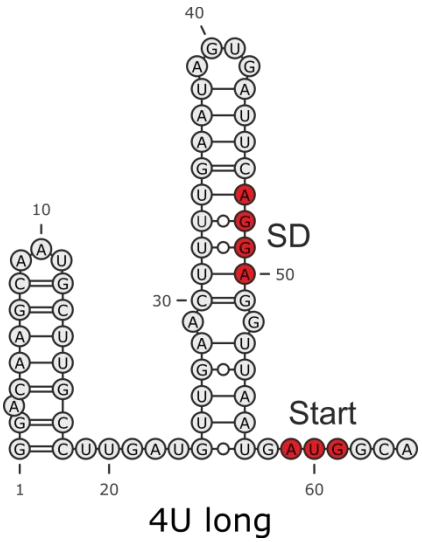
Figure S1

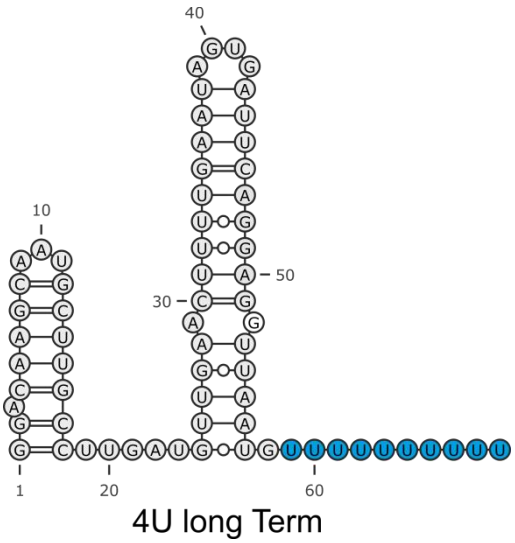
4U long
ΔG: -16.8 kcal/mol

GGACAAGCAATGCTTGCCTTGATGTTGAACTTTTGAATAGTGATTCAGGAGGTTAATGATGGCA



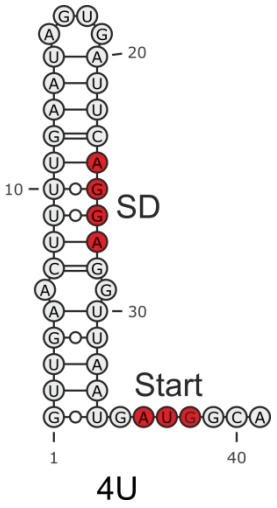
4U long Term
ΔG: -18.6 kcal/mol

GGACAAGCAATGCTTGCCTTGATGTTGAACTTTTGAATAGTGATTCAGGAGGTTAATGTTTTTTTTTT



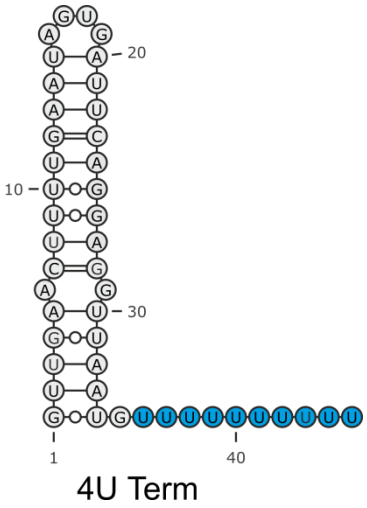
4U
ΔG: -9.7 kcal/mol

GTTGAACTTTTGAATAGTGATTCAGGAGGTTAATGATGGCA



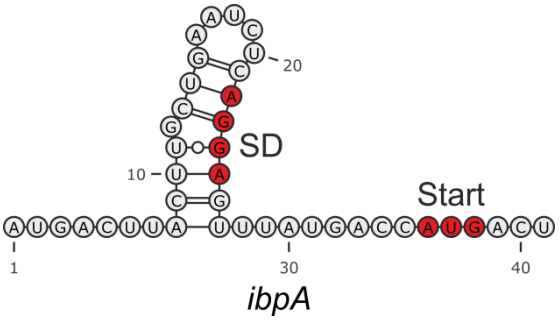
4U Term
ΔG: -9.7 kcal/mol

GTTGAACTTTTGAATAGTGATTCAGGAGGTTAATGTTTTTTTTTT



ibpA
ΔG: -3.8 kcal/mol

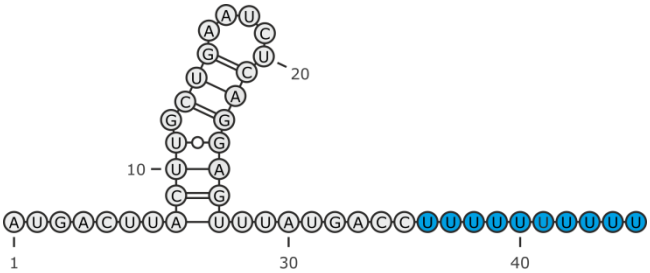
ATGACTTACTTGCTGAATCTCAGGAGTTTATGACCATGACT



ibpA

***ibpA* Term**
ΔG: -3.8 kcal/mol

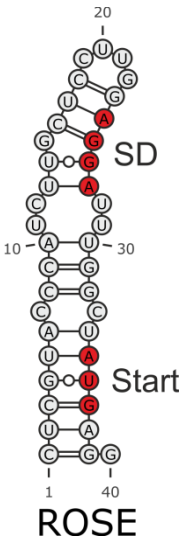
ATGACTTACTTGCTGAATCTCAGGAGTTTATGACCTTTTTTTTTT



ibpA Term

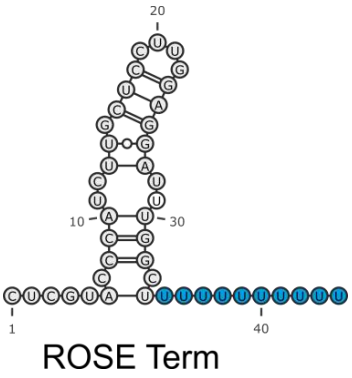
ROSE
ΔG: -14.4 kcal/mol

CTCGTACCCATCTTCTCTTGGAGGATTTGGCT**ATG**AGG



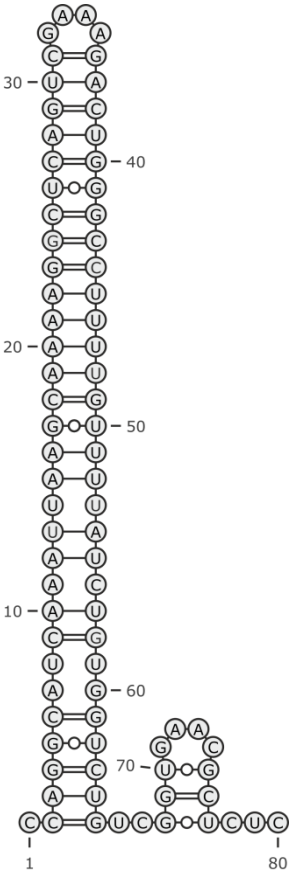
ROSE Term
ΔG: -6 kcal/mol

CTCGTACCCATCTTCTCTTGGAGGATTTGGCTTTTTTTTTTT



***rrnB* T1**
ΔG: -37.6 kcal/mol

CCAGGCATCAAATTAAGCAAAAGGCTCAGTCGAAAGACTGGGCCTTTTGTTTTATCTGT
GGTCTGTCGGTGAACGCTCTC



rrnB T1

Figure S1. Sequences and structures of the used RNA thermometers and constructed thermoterms. Structures were predicted with the mfold software[1] and depicted with VARNA.[2] Shine-Dalgarno sequence and the translational start codon (AUG) of the RNATs are labeled in red, the attached poly(U) tail of the potential thermoterms is marked in blue.

Figure S2

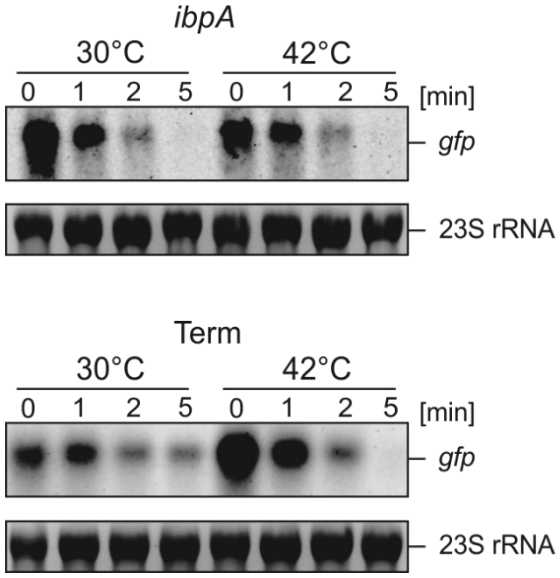


Figure S2. mRNA stability of the *ibpA* RNA thermometer (top) and the *ibpA* thermoterm construct (bottom) at different temperatures. *E. coli* cells harboring translational *gfp* fusions were grown at 30°C to OD600 o.5, induced with 0.01% L-arabinose and heat-shocked to 42°C for 30 min. Rifampicin was added to block transcription and samples were taken at the indicated time points. Transcript levels were detected with a probe againsg the *gfp* mRNA (~730 nt). Half-lives were calculated with the AlphaEaseFC software.

Figure S3

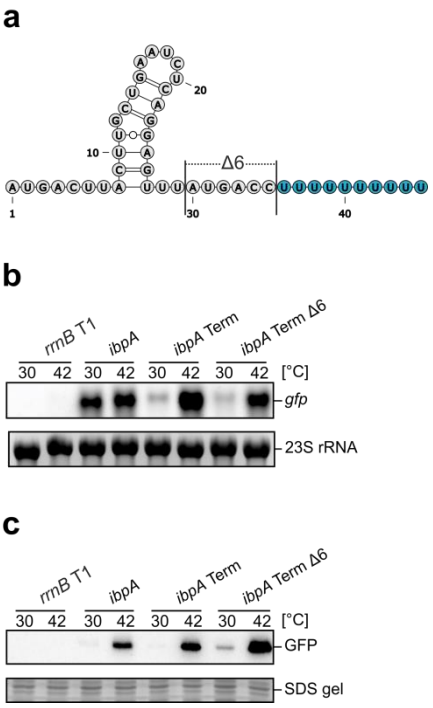


Figure S3. Construction of an *ibpA* Term variant with a directly adjacent poly(U) tail. (**a**) Six nucleotides between the RNAT hairpin and the attached poly(U) tail were deleted via site-directed mutagenesis. Comparative Northern (**b**) and Western (**c**) analyses of reporter gene levels. *E. coli* cells carrying translational *gfp* fusions were grown at 30 °C to an optical density of 0.5, transcription was induced with 0.01 % L-arabinose and cells were heat-shocked for 30 minutes at 42 °C previous to RNA or protein isolation. For detection of RNA levels a probe directed against the *gfp* mRNA was used. Ethidium bromide stained 23S rRNA served as loading control. Synthesized GFP was detected with α-GFP antibody.

Supplementary Table S1: Oligonucleotides used in this study.
T7 promoter sequences for generation of *in vitro* transcripts are written in bold letters. Restriction sites are underlined.

Oligonucleotide	Purpose	Plasmid	Sequence (5' to 3')
1305 rrnBNhe_fw	Cloning of *rrnB* T1 in pBAD2-plasmids	pBO3917, pBO4330	TTGCTAGCCCAGGCATCAAATTAAG
1306 rrnBXho_rv	Cloning of *rrnB* T1 in pBAD2-plasmids	pBO3917, pBO4330	TTCTCGAGGAGAGCGTTCACCGACA
253 agsA_fw	Cloning of *agsA* in pBAD2-plasmids	pBO4381	
254 agsA_rv	Cloning of *agsA* in pBAD2-plasmids	pBO4381	
1799 agsATerm_fw	Cloning of 4U long Term in pBAD2-plasmids	pBO4376, pBO4378	TTTGCTAGCGGACAAGCAATGCTTG
1800 agsATerm_rv	Cloning of 4U long Term and 4U Term in pBAD2-plasmids	pBO4376, pBO4378, pBO4377, pBO4382	TTTCTCGAGAAAAAAAAAACATTAACCTCCTGA
135 miniagsA_fw	Cloning of mini *agsA* in pBAD2-plasmids	pBO4326	
136 miniagsA_rv	Cloning of mini *agsA* in pBAD2-plasmids	pBO4326	
1801 miniTerm_fw	Cloning of 4U Term in pBAD2-plasmids	pBO4377, pBO4382	TTTGCTAGCGTTGAACTTTTGAATAG
1806 ibpA_fw	Cloning of *ibpA* and *ibpA* Term in pBAD2-plasmids	pBO4385, pBO4387, pBO4386, pBO4388, pBO6017	TTTGCTAGCATGACTTACTTGCTGAAT
1807 ibpA_rv	Cloning of *ibpA* in pBAD2-plasmids	pBO4385, pBO4387	TTTGAATTCAGTCATGGTCATAAACTCC
1808 ibpATerm_rv	Cloning of *ibpA* Term in pBAD2-plasmids	pBO4386, pBO4388	TTTCTCGAGAAAAAAAAAAGGTCATAAACTCCT
1796 ROSETerm_fw	Cloning of ROSE and ROSE Term in pBAD2-plasmids	pBO4383, pBO4384, pBO4389, pBO4390	TTTGCTAGCCTCGTACCCATCTTCT
1797 ROSETerm_rv	Cloning of ROSE Term in pBAD2-plasmids	pBO4383, pBO4390	TTTCTCGAGAAAAAAAAAAAGCCAAATCCTCCA
1798 ROSE_rv	Cloning of ROSE in pBAD2-plasmids	pBO4384, pBO4389	TTTGAATTCCCTCATAGCCAAATCCTCC
1852 ibpArep_fw	Site-directed mutagenesis variant U11C/ΔG12	pBO4394, pBO4397	CTCGCTAGCATGACTTACTCCTGAATCTCAGGAGTTTAT
1853 ibpArep_rv	Site-directed mutagenesis variant U11C/ΔG12	pBO4394, pBO4297	ATAAACTCCTGAGATTCAGGAGTAAGTCATGCTAGCGAG

1854 ibpAderep_fw	Site-directed mutagenesis variant C13A	pBO4395, pBO4396	TCGCTAGATGACTTACTTGATGAATCTCAGGAGTTTATG
1855 ibpAderep_rv	Site-directed mutagenesis variant C13A	pBO4395, pBO4296	CATAAACTCCTGAGATTCATCAAGTAAGTCATCTAGCGA
2080 ibpAdrp_fw	Site-directed mutagenesis variant C13G/U14A	pBO6000, pBO6016	CGCTAGCATGACTTACTTGGAGAATCTCAGGAGTTT
2081 ibpAdrp_rv	Site-directed mutagenesis variant C13G/U14A	pBO6000, pBO6016	AAACTCCTGAGATTCTCCAAGTAAGTCATGCTAGCG
2082 ibpAdrp2_fw	Site-directed mutagenesis variant +G12/13	pBO6004, pBO6013	CGCTAGCATGACTTACTTGGCTGAATCTCAGGAGTTTAT
2083 ibpAdrp2_rv	Site-directed mutagenesis variant +G12/13	pBO6004, pBO6013	ATAAACTCCTGAGATTCAGCCAAGTAAGTCATGCTAGCG
2077 MCS_fw	Insertion of 200nts upstream of *rrnB* T1, *ibpA* and *ibpA* Term in pBAD2-*gfp*	pBO6001, pBO6003	TTTGCTAGCAAGGGCGATCGGTGCGGGC
2078 MCS_rv	Insertion of 200nts upstream of *rrnB* T1, *ibpA* and *ibpA* Term in pBAD2-*gfp*	pBO6001, pBO6003	TTTGCTAGCTATGACCATGATTACGAAT
2075 ibpARO_fw	RunOff plasmid	pBO6008, pBO6009	TTT**GAAATTAATACGACTCACTATAGGG**ATGACTTACTTGCTGAATCT
2076 ibpARO_rv	RunOff plasmid	pBO6008	TTTGATATCAGTCATGGTCATAAACTCCTGAG
2077 TermRO_rv	RunOff plasmid	pBO6009	TTTGATATCAAAAAAAAAAGGTCATAAACTCCTG
2079 Termopt_rv	*ibpA* Term variant Δ6	pBO6017	TTTCTCGAGAAAAAAAAAAACTCCTGAGATTCA
1724 GFPprobe_fw	RNA probe for northern blot analysis	--	CTTCTTCAAGGATGACGGGAACTACA
1725 GFPprobe_rv	RNA probe for northern blot analysis	--	**GAAATTAATACGACTCACTATAGGG**GACAGGGCCATCGCCGATGGGCGT

Supplementary Table S2: Plasmids used in this study

Plasmid	Nomenclature	Relevant Characteristics	Reference or source
pK18		Cloning vector, Km^r	[3]
pBAD2-*bgaB*		plasmid for translational *bgaB* reporter gene fusions with arabinose inducible promoter; Amp^r	[4]
pBO3390		pBAD2-*bgaB* with Gentamicin cassette and Shine Dalgarno sequence; plasmid for translational *bgaB* reporter gene fusions with arabinose inducible promoter; Amp^r	[5]
pBO4391		pBAD2-*gfp* with Gentamicin cassette and Shine Dalgarno sequence; plasmid for translational *gfp* reporter gene fusions with arabinose inducible promoter; Amp^r	This study
pBO3917	*rrnB*T1	pBAD2-*bgaB*; translational *bgaB*-fusion *rrnB* T1 (Terminator of ribosomal RNA operon)	This study
pBO4330	*rrnB*T1	pBAD2-*gfp*; translational *gfp*-fusion *rrnB* T1 (Terminator of ribosomal RNA operon)	This study
pBO2153	4U long	pBAD2-*bgaB*; translational *bgaB*-fusion *agsA* 5'UTR	Aschke, unpublished
pBO4381	4U long	pBAD2-*gfp*; translational *gfp*-fusion *agsA* 5'UTR	This study
pBO4376	4U long Term	pBAD2-*bgaB*; translational *bgaB*-fusion *agsA* 5'UTR + 10 additional Ts	This study
pBO4378	4U long Term	pBAD2-*gfp*; translational *gfp*-fusion *agsA* 5'UTR + 10 additional Ts	This study
pBO2174	4U	pBAD2-*bgaB*; translational *bgaB*-fusion mini *agsA* 5'UTR	[6]
pBO4326	4U	pBAD2-*gfp*; translational *gfp*-fusion mini *agsA* 5'UTR	This study
pBO4377	4U Term	pBAD2-*bgaB*; translational *bgaB*-fusion mini *agsA* 5'UTR + 10 additional Ts	This study
pBO4382	4U Term	pBAD2-*gfp*; translational *gfp*-fusion mini *agsA* 5'UTR + 10 additional Ts	This study
pBO4385	*ibpA*	pBAD2-*bgaB*; translational *bgaB*-fusion mini *ibpA*	This study
pBO4387	*ibpA*	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA*	This study
pBO4394	*ibpA* rep	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA*; variant U11C/ΔG12	This study
pBO4395	*ibpA* derep C13A	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA*; variant C13A	This study
pBO6000	*ibpA* derep C13G/U14A	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA*; variant C13G/U14A	This study
pBO6004	*ibpA* derep +G12/13	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA*; variant +G12/13	This study
pBO4386	*ibpA* Term	pBAD2-*bgaB*; translational *bgaB*-fusion mini *ibpA* 5'UTR + 10 additional Ts	This study
pBO4388	*ibpA* Term	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA* 5'UTR + 10 additional Ts	This study
pBO4397	*ibpA* Term rep	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA* 5'UTR + 10 additional Ts; variant U11C/ΔG12	This study
pBO4396	*ibpA* Term derep C13A	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA* 5'UTR + 10 additional Ts; variant C13A	This study
pBO6016	*ibpA* Term derep C13G/U14A	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA* 5'UTR + 10 additional Ts; variant C13G/U14A	This study
pBO6013	*ibpA* Term derep +G12/13	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA* 5'UTR + 10 additional Ts; variant +G12/13	This study
pBO4384	ROSE	pBAD2-*bgaB*; translational *bgaB*-fusion mini ROSE 5'UTR	This study
pBO4389	ROSE	pBAD2-*gfp*; translational *gfp*-fusion mini ROSE 5'UTR	This study

pBO4383	ROSE Term	pBAD2-*bgaB*; translational *bgaB*-fusion mini ROSE 5'UTR + 10 additional Ts	This study
pBO4390	ROSE Term	pBAD2-*gfp*; translational *gfp*-fusion mini ROSE 5'UTR + 10 additional Ts	This study
pBO6001	*rrnB* T1 +200nt	pBAD2-*gfp*; translational *gfp*-fusion *rrnB* T1 (Terminator of ribosomal RNA operon) + 200nt upstream	This study
pBO6003	*ibpA* Term +200nt	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA* 5'UTR + 10 additional Ts; + 200nt upstream	This study
pBO6008		mini *ibpA* 5'UTR + T7 promoter	This study
pBO6009		mini *ibpA* 5'UTR + 10 additional Ts + T7 promoter	This study
pBO6017	*ibpA* Term Δ6	pBAD2-*gfp*; translational *gfp*-fusion mini *ibpA* 5'UTR + 10 additional Ts; Δ30-35	This study

References

1. Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res 31*, 3406-3415.
2. Darty, K., Denise, A., and Ponty, Y. (2009) VARNA: Interactive drawing and editing of the RNA secondary structure. *Bioinformatics 25*, 1974-1975.
3. Pridmore, R. D. (1987) New and versatile cloning vectors with kanamycin-resistance marker. *Gene 56*, 309-312.
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