

Supplementary Information Appendix

## Regulation of Stringent Factor by Branched-Chain Amino Acids

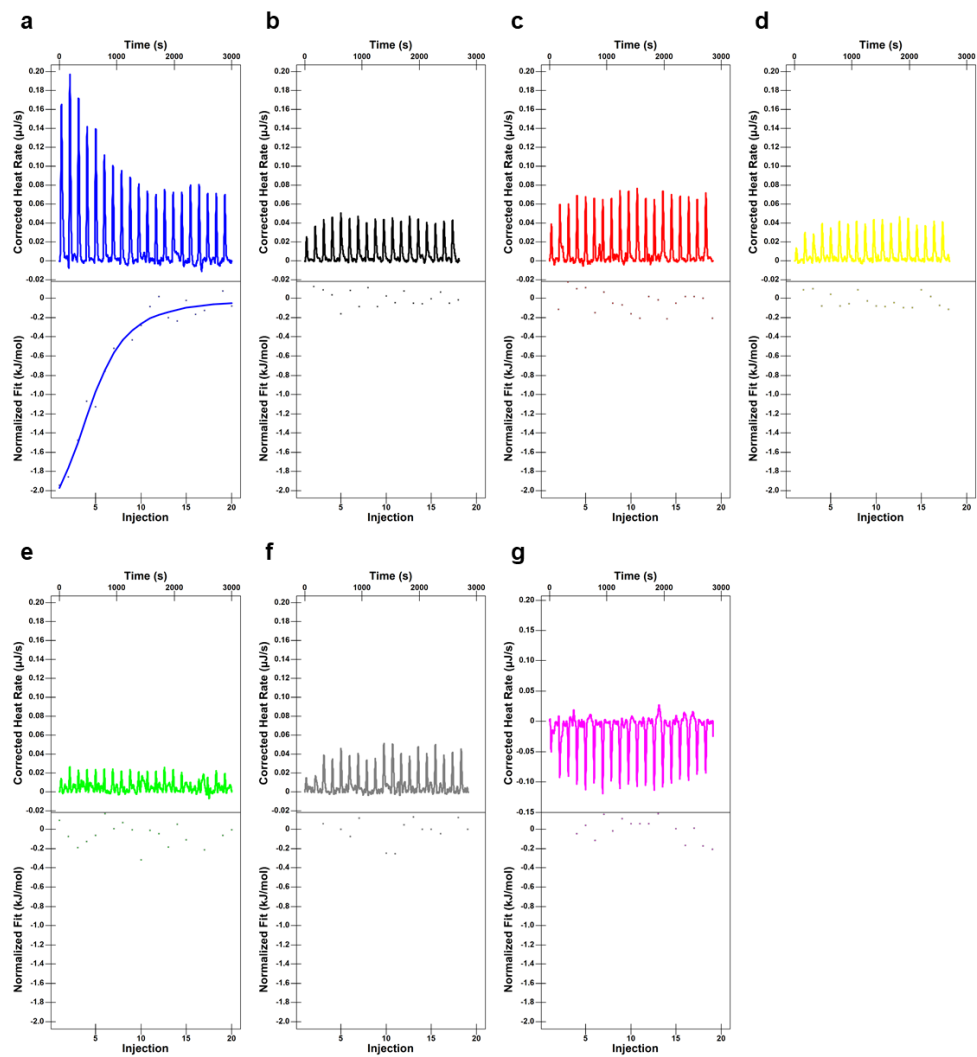
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**Fig. S1.** ITC experiments between 20 amino acids and RcRel<sub>385-705</sub>. 20 amino acids (10 mM) were divided into 7 groups (A: Val, Leu, Ile; B: Ala, Met, Pro; C: Asp, Glu; D: Gly, Phe, Trp; E: Tyr, Asn, Gln; F: His, Arg, Lys; G: Ser, Thr, Cys) to titrate RcRel<sub>385-705</sub>.

**Table S1.** Effect of different divalent cations on hydrolase activity of RcRel.

Cation	RcRel hydrolase activity
Zn <sup>2+</sup>	+
Ni <sup>2+</sup>	-
Mn <sup>2+</sup>	+++
Mg <sup>2+</sup>	+++
Cu <sup>2+</sup>	-
Co <sup>2+</sup>	++
Ca <sup>2+</sup>	+

**Table S2. Kinetics parameters of RcRel hydrolase with branched-chain amino acids.**

	Vmax ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ )	Km ( $\mu\text{M}$ )
Apo	3.5 $\pm$ 0.2	63.0 $\pm$ 12.8
+Val	20.8 $\pm$ 0.6	70.5 $\pm$ 6.5
+Ile	17.5 $\pm$ .07	62.0 $\pm$ 7.7
+Leu	5.3 $\pm$ 0.2	69.9 $\pm$ 7.3

Data represented as best-fit values to Michaelis-Menton equation  $\pm$  standard errors

**Table S3.** *In vivo* concentrations of amino acids in *R. capsulatus* cells grown in either PY or RCV medium. Data were represented as mean (n=3)  $\pm$  standard errors.

	[In PY] ( $\mu$ M)	[In RCV] ( $\mu$ M)	[In PY] / [In RCV]
Ala	505.1 $\pm$ 19.2	514.1 $\pm$ 74.2	1.0
Gly	668.6 $\pm$ 72.5	636.3 $\pm$ 92.7	1.1
<b>Val</b>	<b>3242.4 <math>\pm</math> 140.2</b>	<b>59.6 <math>\pm</math> 6.7</b>	<b>54.4</b>
<b>Leu</b>	<b>1509.8 <math>\pm</math> 102.2</b>	<b>55.6 <math>\pm</math> 7.6</b>	<b>27.2</b>
<b>Ile</b>	<b>656.2 <math>\pm</math> 54.6</b>	<b>32.9 <math>\pm</math> 3.2</b>	<b>19.9</b>
Pro	228.2 $\pm$ 15.4	147.3 $\pm$ 7.0	1.5
Met	107.7 $\pm$ 27.1	47.6 $\pm$ 7.7	2.3
Ser	163.8 $\pm$ 19.4	108.7 $\pm$ 9.2	1.5
Thr	1305.9 $\pm$ 34.1	391.7 $\pm$ 48.0	3.3
Phe	531.9 $\pm$ 65.5	119.4 $\pm$ 16.4	4.5
Asp	307.9 $\pm$ 36.4	180.6 $\pm$ 14.9	1.7
Glu	2108.7 $\pm$ 88.2	8951.1 $\pm$ 946.7	0.2
Lys	4163.9 $\pm$ 1494.9	644.0 $\pm$ 366.7	6.5
His	283.2 $\pm$ 91.4	137.3 $\pm$ 2.8	2.1
Tyr	165.7 $\pm$ 17.9	52.4 $\pm$ 2.2	3.2

**Table S4.** Thermodynamic parameters obtained from Isothermal Titration Calorimetry experiments.

Protein	Ligand	$\Delta H$ (kJ/mol)	$T\Delta S$ (kJ/mol)	$\Delta G$ (kJ/mol)
<i>R. capsulatus</i> Rel	Val	-14.07	13.90	-27.97
<i>R. capsulatus</i> Rel	Ile	-7.32	13.89	-21.21
<i>E. coli</i> RelA	Val	-23.53	8.14	-31.67
<i>R. centenum</i> Rel	Val	-23.41	-2.46	-20.95
<i>R. centenum</i> Rel	Ile	-11.43	5.69	-17.12
<i>B. subtilis</i> Rel	Leu	-9.19	11.62	-20.81
<i>S. aureus</i> Rel	Leu	-14.09	5.94	-20.03

**Table S5:** Primer list

*Rhodobacter capsulatus* SB1003

Rel F NdeI ACTGCATATGATCGATGTCGAAGACCTGA  
Rel 624 F NdeI CCGGTCTACATATGTCGGTGGCGCTGAAGATCACC  
Rel Strep R SacI TGCCTTGAGCTCCCTCActtttcgaactgcgggtggctccagctcgcGGGTTGCGCGACAGGTCCG  
Rel 381 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagGAGATGTATCCGATCAGGT  
Rel R SacI TGCCTTGAGCTCCCTCAGGGTTGCGCGACAGGTC  
Rel N651A mutagenesis F CGGGGCGCAGAAAGCCGCTATCTCCGACCTTCAT  
Rel N651A mutagenesis R ATGAAGGTCGAGATAGCGGCTTCTGCGCCCG  
N651A 1kb F XbaI TCGGTCTAGATGATCACGGCGCCGGCAGC  
N651A 1kb R SacI CAAAGAGCTCAGCAGCCCGCGGCATGGGC

*Escherichia coli* K-12

RelA 400 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagCAGGTCTTGACGACCGGGT  
RelA R SacI CAAATTGAGCTCGGCTAACTCCCGTCAACCGACGCGCG  
SpoT 382 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagGATCTCTCCCGATGAGATT  
SpoT R SacI CAAATTGAGCTCGGCTTAATTCGGTTTCGGGTGACTTT

*Bacillus subtilis* PY79

Rel 388 Strep F NcoI GGAGATATACCATGGcgtagaccaccgcagttcgaagGATTTGTTCTCTGACATGG  
Rel R SacI CAAATTGAGCTCGGCTTAGTTCATGACGCGGCACAG

*Rhodospirillum centenum* SW

Rel 396 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagGAACTGTTCCAGGACAGGTC  
Rel R SacI TGCCTTGAGCTCCCTCAGCGCTTCTTGACGCT

*Deinococcus radiodurans* R1

Rel 454 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagCAGCGGGTGTGGGTCTTCAC  
Rel R SacI TGCCTTGAGCTCCCCTAGGCCGCTTCGCCCA

*Enterococcus faecalis*

Rel 388 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagGAAGACATTTTTAGCGACAA  
Rel R SacI TGCCTTGAGCTCCCTACCATTGGTTCGACGAAC

*Staphylococcus aureus* Newman

Rel 394 Strep F NcoI AGATATACCATGGCGtagaccaccgcagttcgaagAAATATGACTTACAGAGTGAC  
Rel R SacI TGCCTTGAGCTCCCTAGTTCAAACTCTTGTTAC

*Vibrio harveyi*

RelA 396 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagCAGGTATTTGATGACCGGT  
RelA R SacI CAAATTGAGCTCGGCTTAGCCTAAGCGCTTACCGA  
SpoT 383 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagGATCTTTCCAGATGAGATT  
SpoT R SacI CAAATTGAGCTCGGCTTAGTCTTTCTGCTGAC

*Pseudomonas aeruginosa* PAO1

RelA 410 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagGATATCGAGCCGGACAGGGTC  
RelA R SacI CAAATTGAGCTCGGCTCAAGGCGTACGGTTGCGCG  
SpoT 382 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagGACCTGTTCCCGACGAGGTC  
SpoT R SacI CAAATTGAGCTCGGCTCAGCTACGCGGGTGA

*Caulobacter crescentu s* CB15

Rel 408 Strep F NdeI GGAGATATACATATGtgagaccaccgcagttcgaagGAGATGTTCTCGACCAGGTT  
Rel R EcoRI TGCCTTGAATCCCTACCCCGCTCCGTCCA

Restriction sites are highlighted in red.

Strep tag sequences are in lower case.