Supporting Information:

Thermodynamics of Coupled Folding in the Interaction of Archaeal RNase P Proteins RPP21 and RPP29

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Supporting Information

Table S.1

Table S.1: Effect of ion linkage on thermodynamics of RPP21-RPP29 interactions at 10°C and 55°C for titrations of RPP21 into RPP29 in 20 mM cacodylate, pH 6.7, 0.3 mM $ZnCl_2$, 3 mM NaN_3.[a]

10°C

[KCl] (mM)	I[b]	N	K_A (/10^6)	ΔG	ΔH	TΔS
10	31.8	0.956 ± 0.06	9.17 ± 2.36	-9.00 ± 0.14	17.10 ± 0.53	26.08 ± 0.40
50	71.8	0.992 ± 0.03	87.57 ± 12.60	-10.27± 0.08	17.38 ± 1.51	27.64 ± 1.53
100	121.8	0.993 ± 0.02	198.00 ± 30.51	-10.73 ± 0.08	19.15 ± 0.88	29.90 ± 0.86
150	171.8	0.964 ±0.02	240.67 ± 14.74	-10.85 ± 0.03	19.42 ± 1.75	30.28 ± 1.72

55°C

[KCl] (mM)	I[b]	N	K_A (/10^6)	ΔG	ΔH	TΔS
10	31.8	0.947 ± 0.01	1.72 ± 0.20	-9.35 ± 0.07	-39.61 ± 1.48	-30.24 ± 1.54
50	71.8	0.946 ± 0.01	18.00 ± 0.96	-10.88 ± 0.03	-38.79 ± 0.91	-27.89 ± 0.93
100	121.8	1.030 ± 0.08	43.27 ± 4.33	-11.45 ± 0.07	-33.98 ± 2.14	-22.51 ± 2.07
150	171.8	0.983 ± 0.10	75.63 ± 2.63	-11.82 ± 0.02	-32.91 ± 1.64	-21.07 ± 1.67

[a] Binding parameters are N (number of RPP21 binding per RPP29), K_A (association equilibrium constant) in M^{-1}, and ΔG, ΔH and TΔS in kcal mol^{-1}. Reported uncertainties are the standard deviation of three replicates.

[b] Ionic strength of the solutions, $I = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2$, where c is the concentration of charged species i, z is the charge on the species; at pH 6.7, 20 mM cacodylate is 14.9 mM ionic strength (pKa 6.4).

Table S.2

Table S.2: Effect of salt on the temperature-dependence of ΔH[a]

	Cacodylate[b]	10 mM KCl		150 mM KCl	
Temp	ΔH_{ion}	ΔH_{obs}	ΔH_{bind}	ΔH_{obs}	ΔH_{bind}
10°C	-0.41	17.10 ± 0.53	16.82 ± 0.53	19.42 ± 1.75	19.14 ± 1.75
15°C	-0.51	11.64 ± 0.21	11.29 ± 0.21	-	-
20°C	-0.61	5.95 ± 0.15[c]	5.53 ± 0.15	8.49 ± 0.12[c]	8.07 ± 0.12
30°C	-0.82	-5.19 ± 0.45[d]	-5.75 ± 0.45	-	-
35°C	-0.92	-10.25 ± 0.25	-10.88 ± 0.25	-9.55 ± 0.17[c]	-10.17 ± 0.17
40°C	-1.03	-15.84 ± 1.27	-16.54 ± 1.27	-	-
45°C	-1.13	-23.53 ± 0.42	-24.30 ± 0.42	-22.06 ± 0.37[c]	-22.83 ± 0.37
55°C	-1.33	-31.98 ± 0.51	-32.89 ± 0.51	-32.91 ± 1.64	-33.81 ± 1.64

[a] Cacodylate buffer ionization enthalpy change (ΔH_{ion}), observed enthalpy change (ΔH_{obs}) and ionization-corrected binding enthalpy (ΔH_{bind}), in kcal mol^{-1}. ΔH_{bind} calculated from equation 6, using a temperature-independent proton linkage number of -0.7, as determined at 55°C, pH 6.7.

[b] The buffer ionization enthalpy calculated based on its published temperature dependence.[30]

[c] The reported uncertainties are standard fitting errors from one experiment.

[d] The reported uncertainties are variances from two replicates.

Table S.3

Table S.3: Temperature dependent thermodynamics for titration of RPP29WT into RPP21V14[a]

Temp	N	K_A (/10^6)	ΔG	ΔH	TΔS
5°C	0.970 ± 0.006	5.65 ± 0.57	-8.58 ± 0.06	13.98 ± 0.12	22.55 ± 0.13
15°C	0.972 ± 0.012	11.00 ± 3.10	-9.27 ± 0.16	5.42 ± 0.11	14.69 ± 0.19
35°C	1.010 ± 0.012	7.61 ± 1.80	-9.69 ± 0.14	-13.53 ± 0.24	-3.82 ± 0.28
45°C	0.992 ± 0.007	2.10 ± 0.16	-9.19 ± 0.05	-23.93 ± 0.24	-14.72 ± 0.24
55°C[b]	0.987 ± 0.033	0.36 ± 0.06	-8.34 ± 0.10	-43.18 ± 2.09	-34.77 ± 2.09

[a] Binding parameters are N (number of RPP29WT binding per RPP21V14), K_A (association equilibrium constant) in M^{-1}, and ΔG, ΔH and TΔS in kcal mol^{-1}. Experiments were conducted in 20 mM cacodylate (sodium salt), pH 6.7, 10 mM KCl, 0.03 ZnCl2 and 0.02% NaN_3. Reported uncertainties are standard errors from least squares fit of the thermograms to a single binding site model with the Origin (V.7 SR4) software package.

[b] Since ΔH at 55°C decreased significantly in a manner inconsistent with the linear relationship observed in the rest of the data, we chose to exclude ΔH at 55°C when determining the temperature-independent ΔC_p within the range from 5°C to 45°C.

Supplementary Figures

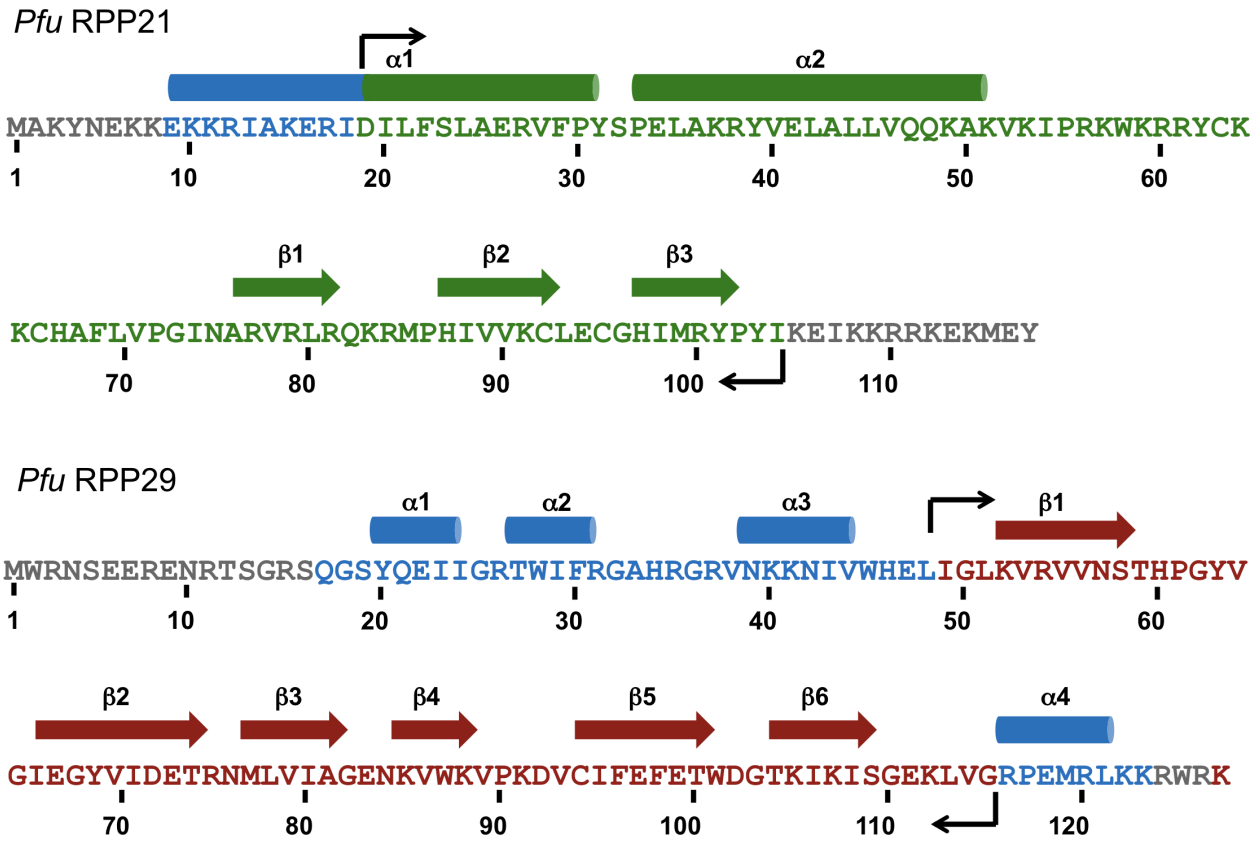

Figure S 1. Primary sequence and coupled folding of RPP21 and RPP29. Secondary structure elements are shown above the primary sequences of *Pfu* RPP21 (NCBI accession no. NP_579342) and RPP29 (NCBI accession no. NP_579545). Arrows indicate the structured cores of RPP21 (green) and RPP29 (red) based on NMR studies, and the segments that only become ordered in the presence of its binding partner are highlighted in blue. Residues not observed in free and the bound states are labeled in grey. Color scheme matches that of Figure 1.

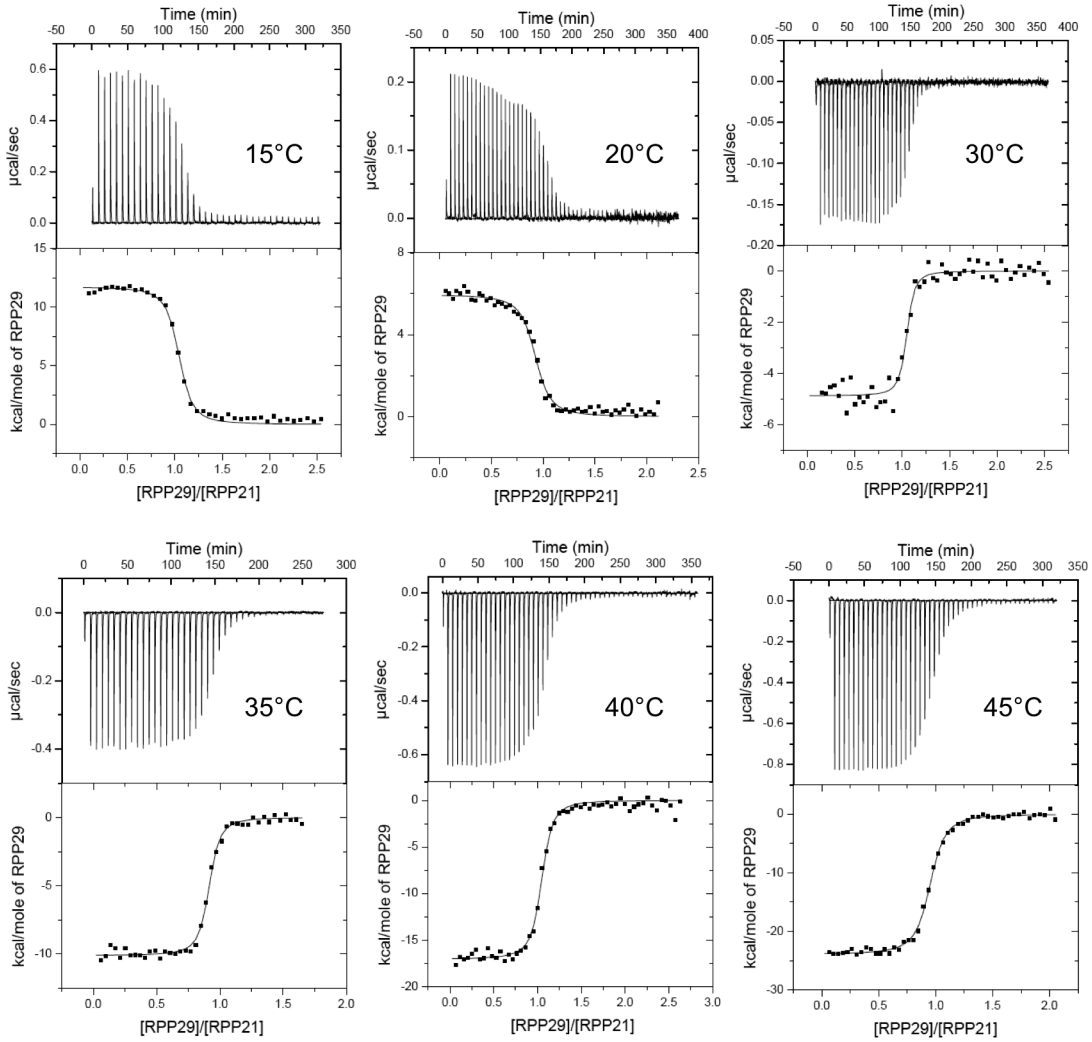

Figure S 2: Representative isotherms for titrations of RPP29 into RPP21 in standard ITC buffer from 15°C to 45°C with an increase of 5°C. Titration was not performed at 25°C because of the small ΔH observed at that temperature (T_H = 25.45°C). Best fit parameters are shown in Table 1.

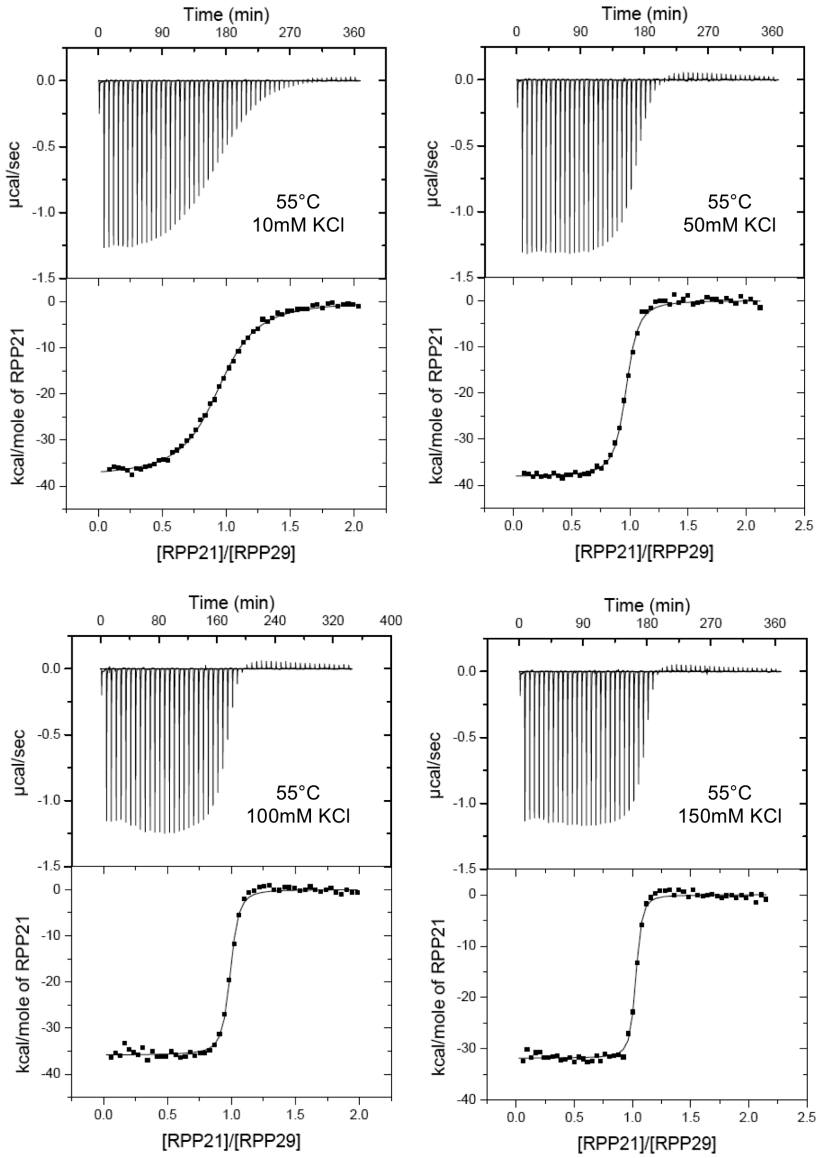

Figure S 3: Representative isotherms for titrations of RPP21 to RPP29 at 55°C in the standard ITC buffer with 10 mM, 50 mM, 100 mM and 150 mM of KCl. Best fit parameters are shown in Table S.1.

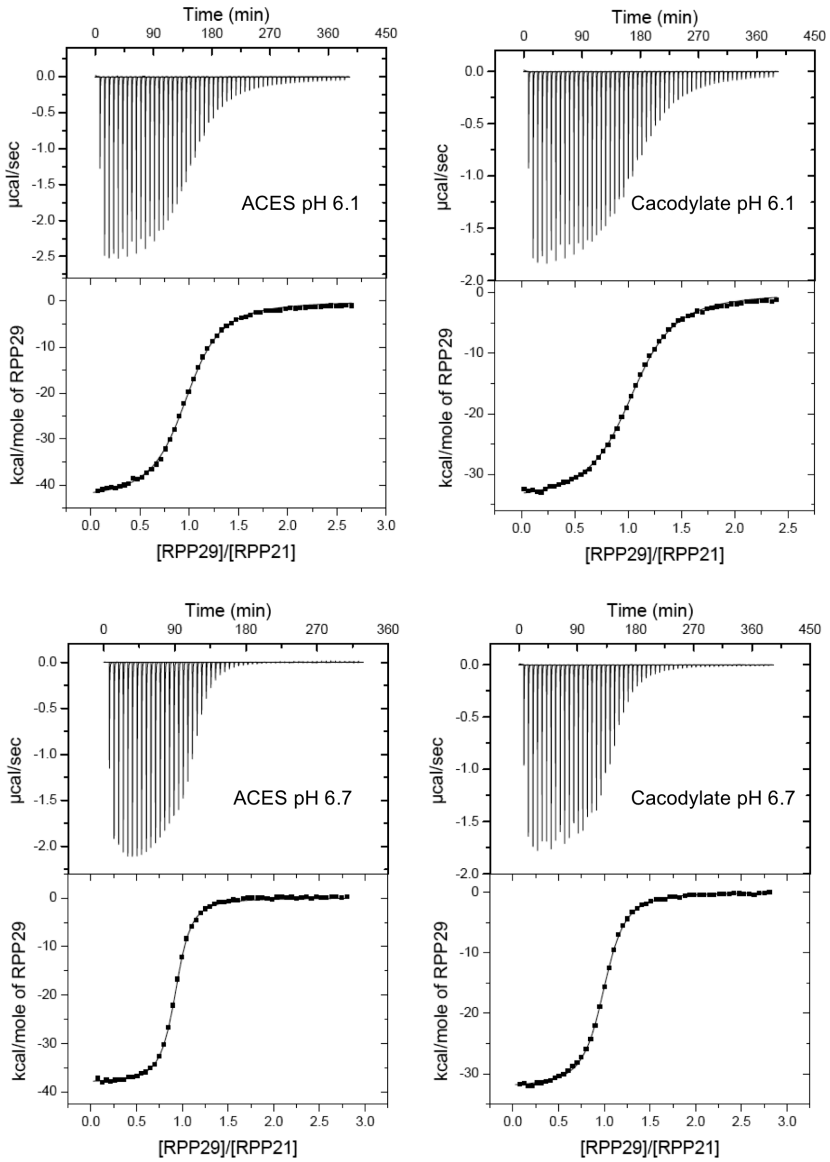

Figure S 4: Representative isotherms of RPP21-RPP29 binding at 55°C in buffers with different ΔH_{ion} at pH 6.1 and pH 6.7. All conditions consist of 20 mM buffering component (cacodylate or ACES), 10 mM KCl, 0.3 mM $ZnCl_2$ and 0.02% NaN_3. Best fit parameters are shown in Table 2.

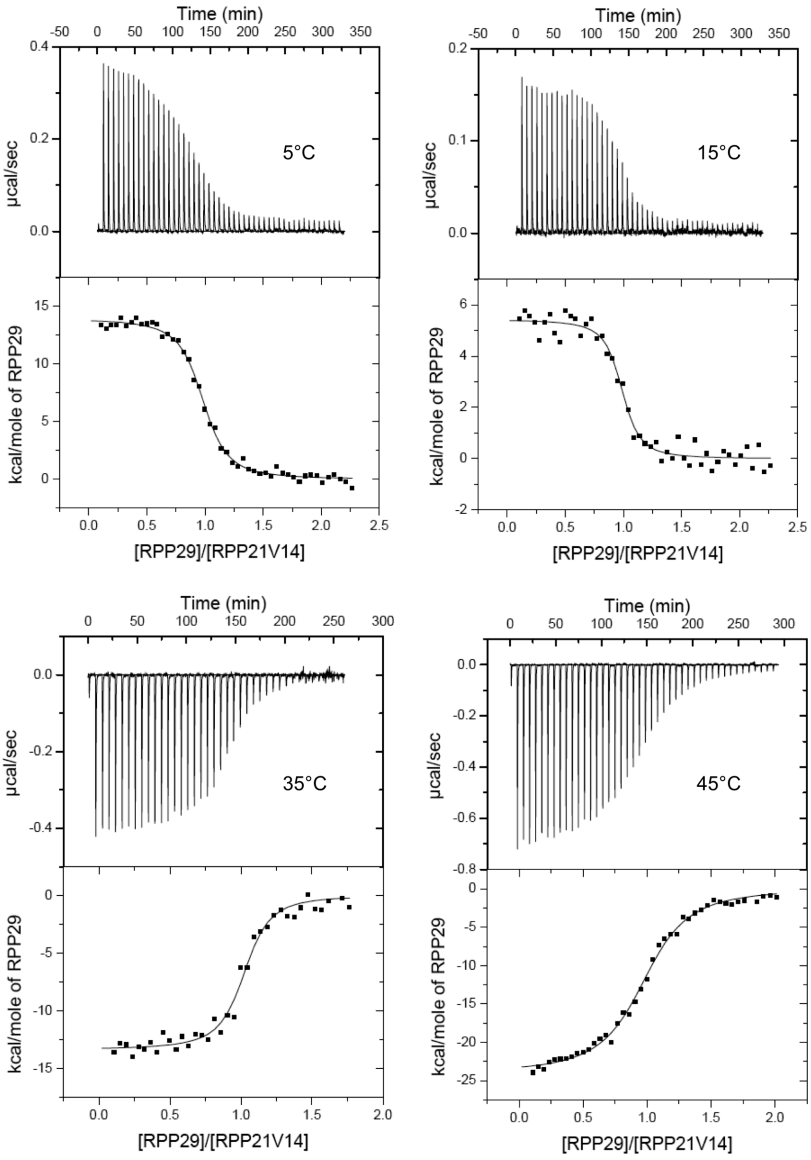

Figure S 5: Representative isotherms for titrations of RPP29wt to RPP21V14 in the standard ITC buffer at 5°C, 15°C, 35°C, and 45°C. Best fit parameters are shown in Table S.3

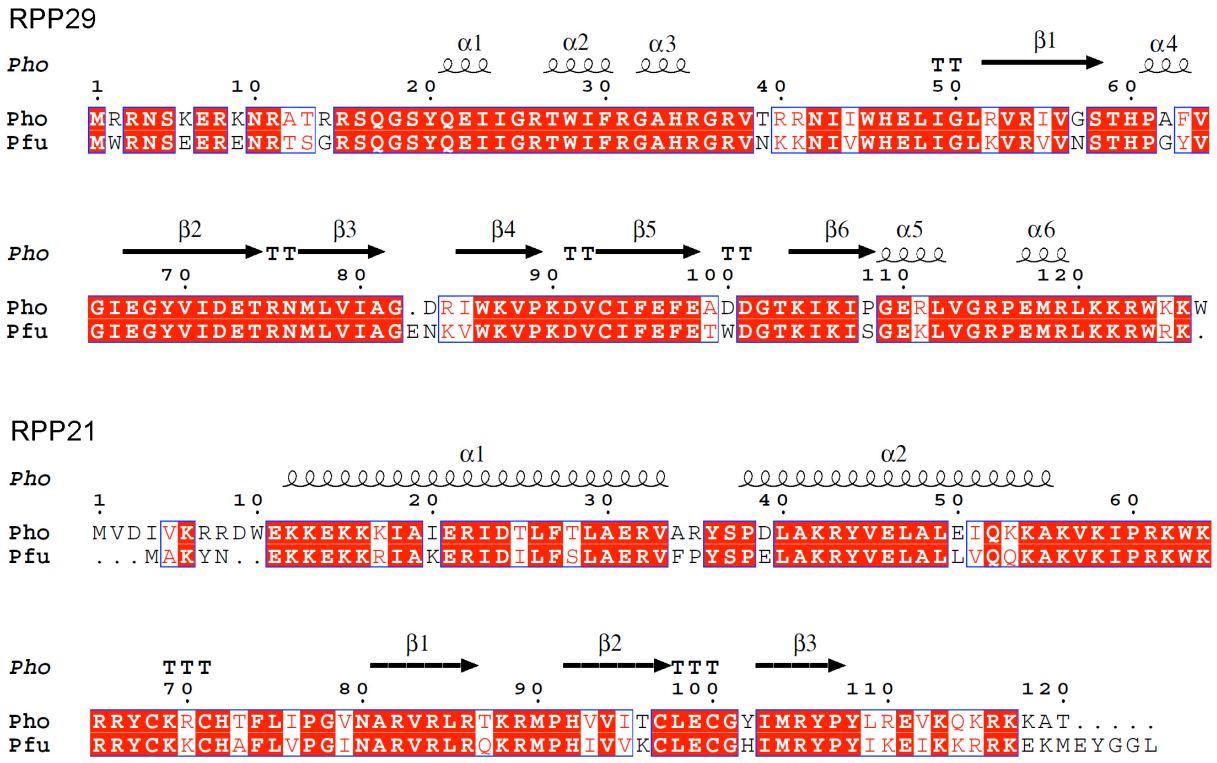

Figure S 6**:** Alignment of *Pyrococcus horikoshii* RPP21 and RPP29 sequences with *Pyrococcus furiosus* homologs. The alignment was generated with CLUSTALW and illustrated using ESPRIPT2.2. The red letters indicate a global similarity score of 0.7, and red boxed letters indicate invariant residues. *Pyrococcus horikoshii* secondary structural elements, from PDB ID: **2ZAE**, http://dx.doi.org/10.2210/pdb2zae/pdb, are shown on top of the sequence.