Supporting information for:

Deciphering the mechanism of thermodynamic accommodation of telomeric oligonucleotide sequences by the *S. pombe* Protection of Telomeres 1 (Pot1pN) protein (†).

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<u>Supplemental Table 1:</u> Solvent accessible surface area (ASA) and heat capacity change (ΔCp) for selected ssDNA-protein interactions.

Protein/DNA [PDB aq. #]	Polarity	Accessible Surface Area (ASA, Å^2 mol^{-1})				ΔCp_{dehyd} (cal K^{-1}mol^{-1}) (normalized)[c]	ΔCp_{obs} (cal K^{-1} mol^{-1})	ΔCp_{excess} (cal K^{-1} mol^{-1})
		Complex	Free DNA	Free protein	ΔASA			
POT1pN/Tel6 (this study) (*1*), [1QZH]	Polar	3509.4	768.9	3399.4	658.9	-116 (-0.177)	-372	-256
	Apolar	5401.5	651.3	5400.8	-650.1			
POT1pN/Tel6[a] (this study) (*1*), [1QZH]	Polar	3509.4	1199.4 **1090**	3399.4	-1089.4 **-980**	-193.0 (-0.178)	-372	-179
	Apolar	5401.5	1080.3	5400.8	-1079.7			
*h*POT1DBD/Tel10 (*2*), [1XJV]	Polar	5840.5	1368.9	5358.7	-1368.9	-206 (-0.158)	n.d.	---
	Apolar	9306.6	1244.0	9383.4	-1244			
Cdc13 DBD/Tel11 (*3*) [1S40]	Polar	5242.6	1759.5	4626	-1142.9	-334 (-0.249)	n.d.	---
	Apolar	5958.3	1219.2	6284.4	-1545.3			
TEBPα/Tel8 (*4, 5*), [1KIX]	Polar	9287.7	943.7	9337	-993	-216 (-0.251)	n.d.	---
	Apolar	12676..3	815.4	12676.3	-726			
TEBPαβ(full length)/Tel15 (*6*), [2I0Q]	Polar	13424.8	1676.2	13137.3	-1388.7	-387 (-0.242)	-305 -2315	-1928
	Apolar	17499.8	1305.9	18011.6	-1817.7			
TEBPαN/Tel8[b] (*5, 7*), [1K8G]	Polar	5554.3	653.1	5469.9	-568.7	-110 (-0.203)	-169 -308 (for 11mer)	---
	Apolar	7761.4	505.0	7771.2	-514.8			
TraI36/F-TAM **data from structure of free protein in bold** (*8-10*), [2A0I, 1P4D]	Polar	5984.9	1461.1	5870.2 **(5708.8)**	-1346.4 **(-1185)**	-476.8 (-0.278)		
	Apolar	8313	1247.4	9144.7 **(7997.9)**	-2079.1 **(-932.3)**	**-132.4 (-0.125)**	-3300	-2823 **-3168**
DNA-1/dT5 (*11, 12*), [1I8M]	Polar	14080.1	996.2	13913	-829.1	-105.6 (-0.107)	0	+105.6
	Apolar	23628.1	1046.9	23722	-1140.8			

The program GetArea (http://pauli.utmb.edu/cgi-bin/get_a_form.tcl) was used to calculate the polar and apolar accessible surface area (ASA).

$\Delta Cp_{dehyd} = (0.32*\Delta ASA_{apolar})-(0.14*\Delta ASA_{polar})$ (*13*)

$\Delta Cp_{excess} = \Delta Cp_{obs} - \Delta Cp_{dehyd}$

[a] Tel6 in an extended conformation modeled in X-PLOR using standard parameter settings (*14*)

[b] Only 5 DNA residues seen in structure

[c] Normalized for the interfacial surface area ($[\Delta ASA_{polar}+\Delta ASA_{apolar}]/2$) of the complex; cal K^{-1} mol^{-1} Å^2

Supplemental Table 2: ***^{1}HN ^{15}N ^{13}CO ^{13}Cα and ^{13}Cβ protein backbone resonances assignments for the Pot1pN/d(GGTTAC) complex.***

Residue Number	Residue Name	^{1}HN (ppm)	^{15}N (ppm)	^{13}CO (ppm)	^{13}Cα (ppm)	^{13}Cβ (ppm)
1	Met	----	----	----	----	----
2	Arg	----	----	173.87	56.90	31.08
3	Gly	110.99	8.60	176.12	45.50	N/A
4	Ser	115.27	8.22	174.37	58.54	64.25
5	His	----	----	----	----	----
6	His	----	----	----	----	----
7	His	----	----	----	----	----
8	His	----	----	----	----	----
9	His	----	----	----	----	----
10	His	----	----	175.12	56.18	30.24
11	Gly	110.53	8.54	174.12	45.66	N/A
12	Ser	115.94	8.34	174.65	58.80	64.25
13	Met	121.96	8.52	176.47	56.04	33.17
14	Gly	109.74	8.36	173.99	45.72	N/A
15	Glu	120.13	8.33	176.06	56.95	30.74
16	Asp	120.55	8.43	175.79	54.73	41.45
17	Val	119.88	7.90	175.86	62.43	33.28
18	Ile	123.37	8.09	174.77	61.80	39.47
19	Asp	123.20	7.83	176.33	53.46	42.58
20	Ser	114.90	8.46	175.54	61.32	63.38
21	Leu	124.39	7.97	179.03	58.09	41.85
22	Gln	119.50	8.25	178.02	58.50	29.37
23	Leu	120.23	8.19	177.70	58.74	41.96
24	Asn	116.22	8.00	177.12	57.21	38.85
25	Glu	119.94	7.80	179.02	59.68	29.90
26	Leu	121.00	7.92	178.03	58.43	38.68
27	Leu	115.05	8.55	180.06	57.85	41.97
28	Asn	116.53	8.15	176.00	55.44	38.94
29	Ala	121.22	7.62	178.84	53.69	19.84
30	Gly	107.90	7.88	172.58	47.02	N/A
31	Glu	120.27	7.40	175.05	55.15	32.63
32	Tyr	120.80	8.62	172.18	56.14	41.17
33	Lys	121.05	8.55	176.80	55.47	36.02
34	Ile	121.77	8.25	176.63	61.32	39.65
35	Gly	117.25	9.06	174.83	46.95	N/A
36	Glu	124.29	8.96	176.45	56.67	30.66
37	Leu	120.26	7.65	174.30	54.54	45.15
38	Thr	115.35	8.01	173.87	62.24	69.63
39	Phe	129.68	9.64	173.49	57.56	42.50
40	Gln	116.54	7.56	176.03	53.21	31.57

41	Ser	117.94	9.09	175.21	58.12	65.39
42	Ile	123.80	9.86	178.96	63.78	35.28
43	Arg	120.43	8.26	179.44	60.14	30.72
44	Ser	113.65	7.75	177.36	61.79	63.25
45	Ser	118.47	8.14	173.43	62.43	70.36
46	Gln	116.17	7.47	176.87	56.94	30.34
47	Glu	118.47	7.38	177.38	56.75	30.77
48	Leu	124.36	8.30	178.32	57.46	42.35
49	Gln	119.88	8.71	176.23	56.72	28.89
50	Lys	119.67	7.97	176.40	56.01	32.65
51	Lys	120.93	7.90	176.11	57.08	33.32
52	Asn	117.89	8.89	174.85	54.84	37.79
53	Thr	113.86	7.90	172.15	61.98	71.35
54	Ile	123.06	8.25	176.71	61.30	39.44
55	Val	120.70	9.51	174.33	59.20	36.40
56	Asn	115.95	8.10	173.41	52.13	41.03
57	Leu	120.39	9.33	174.82	53.54	49.04
58	Phe	126.13	9.97	174.78	55.32	40.42
59	Gly	109.85	9.04	170.66	45.97	N/A
60	Ile	119.27	9.81	176.53	56.90	39.00
61	Val	117.00	8.68	173.47	54.93	----
62	Lys	----	----	175.04	57.49	----
63	Asp	114.06	8.07	173.65	54.35	42.72
64	Phe	118.75	8.60	173.01	57.06	40.72
65	Thr	115.25	9.01	172.28	58.62	71.08
66	Pro	----	----	175.02	----	----
67	Ser	113.80	8.39	174.47	58.97	64.12
68	Arg	121.77	8.83	173.17	55.17	32.26
69	Gln	121.35	8.25	176.19	57.13	28.58
70	Ser	122.97	8.00	172.88	61.47	63.23
71	Leu	122.10	9.07	176.69	55.34	43.68
72	His	113.78	6.82	173.19	53.14	32.53
73	Gly	110.93	8.98	176.42	46.58	N/A
74	Thr	116.39	8.99	174.18	62.64	69.08
75	Lys	114.73	8.11	174.90	58.12	28.62
76	Asp	----	----	----	----	----
77	Trp	----	----	174.88	60.17	----
78	Val	120.51	9.50	174.31	58.76	36.64
79	Thr	123.23	8.72	171.21	61.18	70.78
80	Thr	125.18	7.79	173.34	62.33	70.93
81	Val	120.14	9.12	172.32	59.28	36.49
82	Tyr	122.02	8.30	175.23	56.84	39.09
83	Leu	124.74	8.96	174.69	53.88	47.83
84	Trp	----	----	----	----	----
85	Asp	----	----	----	----	----
86	Pro	----	----	176.45	64.48	31.53

87	Thr	110.44	8.48	175.03	63.93	68.38
88	Cys	120.31	7.70	174.25	58.88	29.87
89	Asp	----	----	----	----	----
90	Thr	----	----	----	----	----
91	Ser	----	----	----	----	----
92	Ser	----	----	----	----	----
93	Ile	----	----	----	----	----
94	Gly	----	----	174.16	44.10	N/A
95	Leu	122.63	8.73	174.84	54.39	45.98
96	Gln	129.15	8.77	174.18	56.11	30.85
97	Ile	127.68	8.98	174.19	59.68	41.81
98	His	125.13	8.38	173.25	55.21	33.43
99	Leu	122.02	8.62	173.68	52.34	42.20
100	Phe	118.43	8.43	178.03	56.69	44.66
101	Ser	111.09	9.13	175.40	57.39	65.61
102	Lys	127.14	9.27	177.09	57.96	33.83
103	Gln	117.03	8.22	175.74	55.84	29.64
104	Gly	107.02	7.69	172.22	44.70	N/A
105	Asn	114.61	7.69	174.90	51.96	35.52
106	Asp	123.17	8.12	175.31	52.44	39.86
107	Leu	118.05	6.59	172.87	52.91	41.53
108	Pro	----	----	175.52	63.17	32.31
109	Val	126.24	8.76	174.84	62.26	31.93
110	Ile	128.83	7.73	173.98	61.47	39.77
111	Lys	125.26	8.90	176.56	56.70	35.10
112	Gln	----	----	----	----	----
113	Val	----	----	175.93	63.03	33.22
114	Gly	116.23	8.26	174.38	45.94	N/A
115	Gln	----	----	----	----	----
116	Pro	----	----	174.48	61.97	32.67
117	Leu	125.68	9.48	171.91	54.70	47.45
118	Leu	130.70	9.24	176.55	54.73	46.30
119	Leu	123.82	9.82	173.89	53.53	46.97
120	His	123.91	9.25	174.26	53.71	32.80
121	Gln	116.73	7.66	170.75	59.13	25.61
122	Ile	123.63	8.81	174.71	57.12	38.64
123	Thr	126.15	9.21	174.51	62.66	69.63
124	Leu	126.60	8.66	176.06	53.96	42.91
125	Arg	121.22	9.08	174.97	54.01	33.84
126	Ser	116.99	8.64	174.05	58.78	64.49
127	Tyr	126.76	8.90	173.61	58.10	40.99
128	Arg	124.38	8.67	175.20	58.01	27.56
129	Asp	116.38	8.44	174.78	55.02	40.25
130	Arg	118.84	8.21	174.91	54.89	33.39
131	Thr	118.53	8.59	172.79	63.13	70.22
132	Gln	123.73	9.21	173.03	53.73	33.23

133	Gly	108.88	9.44	171.71	44.13	N/A
134	Leu	121.88	9.16	176.57	52.91	45.52
135	Ser	115.50	8.41	176.76	58.94	63.68
136	Lys	122.60	8.06	180.98	55.10	30.86
137	Asp	124.84	9.80	178.34	58.88	41.47
138	Gln	115.50	8.66	175.36	55.15	27.35
139	Phe	119.61	7.94	174.68	59.47	37.61
140	Arg	120.52	8.63	174.55	53.31	35.95
141	Tyr	117.23	8.90	171.73	56.39	44.14
142	Ala	120.78	8.88	173.48	52.34	24.24
143	Leu	122.39	7.57	174.03	53.71	48.77
144	Trp	125.30	8.47	173.09	58.66	31.93
145	Pro	----	----	----	----	----
146	Asp	----	----	----	----	----
147	Phe	----	----	----	----	----
148	Ser	----	----	174.64	61.06	63.79
149	Ser	116.91	7.53	175.12	58.56	64.34
150	Asn	121.46	8.50	175.57	54.77	38.98
151	Ser	115.05	8.36	174.87	58.42	64.01
152	Lys	123.03	8.55	176.51	56.67	32.72
153	Asp	119.63	8.28	176.39	54.41	41.26
154	Thr	110.30	7.82	173.14	63.87	70.56
155	Leu	121.04	7.77	176.03	53.71	43.31
156	Cys	126.11	8.39	172.11	56.08	27.66
157	Pro	----	----	176.56	62.38	31.58
158	Gln	121.98	8.44	176.55	58.82	----
159	Pro	----	----	----	----	----
160	Met	----	----	----	----	----
161	Pro	----	----	178.12	68.56	32.64
162	Arg	113.56	9.50	174.40	55.20	30.57
163	Leu	120.25	7.07	178.06	55.73	42.89
164	Met	125.23	8.41	174.84	54.44	35.82
165	Lys	125.70	8.41	176.07	56.83	32.38
166	Thr	115.47	8.03	173.89	60.92	71.73
167	Gly	109.09	8.65	175.49	44.89	N/A
168	Asp	123.56	8.88	178.68	57.89	41.33
169	Lys	119.14	8.90	179.70	59.69	32.01
170	Glu	118.59	9.14	177.36	62.36	29.14
171	Glu	119.04	7.94	177.62	60.32	30.14
172	Gln	115.77	8.17	178.32	58.87	28.76
173	Phe	121.61	7.65	175.90	62.65	39.78
174	Ala	120.69	8.13	179.06	55.85	17.30
175	Leu	118.05	8.01	179.16	58.67	42.49
176	Leu	120.63	7.52	178.64	58.12	----
177	Leu	117.96	8.47	178.86	58.57	42.68
178	Asn	116.25	8.19	175.60	----	38.71

179	Lys	----	----	----	----	----
180	Ile	----	----	----	----	----
181	Trp	----	----	----	----	----
182	Asp	----	----	----	----	----
183	Glu	----	----	----	----	----
184	Gln	----	----	----	----	----
185	Thr	----	----	----	----	----
186	Asn	----	----	175.45	54.22	39.07
187	Lys	119.83	7.64	176.43	56.80	32.85
188	His	117.78	8.13	174.83	55.88	29.34
189	Lys	121.61	8.13	176.37	56.85	33.42
190	Asn	119.53	8.47	175.74	53.85	39.36
191	Gly	109.36	8.34	174.17	45.87	N/A
192	Glu	120.39	8.19	176.38	56.88	30.70
193	Leu	122.56	8.20	176.37	55.65	42.64
194	Leu	122.77	8.16	177.05	55.36	42.89
195	Ser	116.44	8.25	174.71	58.49	64.19
196	Thr	115.30	8.16	173.86	61.88	70.16
197	Ser	123.21	7.95	178.54	60.37	65.22

(----) – Resonance assignments have not been determined for these atoms.
N/A – Cβ resonance assignments are not applicable for glycine residues.

Supplemental Table 3: ***Individual nucleotide sugar H1′, base H5/6/8 and methyl base H7* resonance assignments for free and Pot1pN bound d(GGTTAC) .***

Nucleotide	Atom Name/Position	Free d(GGTTAC) ^{1}H (ppm)	Pot1pN-Bound d(GGTTAC) ^{1}H (ppm)
G1	H1′	6.19	5.78
	H8	8.09	7.91
G2	H1’	7.98	6.06
	H8	7.98	8.04
T3	H1′	6.12	6.13
	H6	7.48	7.43
	H7*	1.72	2.15
T4	H1′	5.99	5.58
	H6	7.37	8.09
	H7*	1.80	1.65
A5	H1′	6.33	5.35
	H8	8.33	8.14
C6	H1′	5.99	5.99
	H6	7.71	7.31
	H5	5.86	5.14

Supplemental Results

Thermal stability of free and d(GGTTAC) bound Pot1pN

The thermal stability of free d(GGTTAC), free Pot1pN and Pot1pN/d(GGTTAC) complex in solution was measured using differential scanning calorimetry (DSC). Thermograms of buffer-corrected protein scans in Supplemental Figure 1 show a 5.3°C increase (from 51.9 to 57.2°C) in the mid point transition temperature in the Pot1pN/d(GGTTAC) complex compared to the free Pot1pN. Differential scanning rates (30, 60 and 90 °C/hour) did not significantly alter the midpoint of transition (data not shown), suggesting that the apparent T_m closely approximates the true T_m. A shallow pre-transition baseline of heat capacity change was observed for both free Pot1pN and the Pot1pN/d(GGTTAC) complex, suggesting the presence a stably folded protein/complex prior to the transition. This data is consistent with the observation by ITC that the binding enthalpy varies linearly with temperature, suggesting minimal contributions from folding to the measured binding enthalpy. The temperature dependence of free d(GGTTAC) monitored by DSC yields a shallow thermogram with no distinct transition over the measured temperature range (5°C to 95°C) (data not shown), suggesting that the free DNA is not structured in solution.

Figure Legend

Supplemental Figure 1. DSC thermograms of 70 μM free Pot1pN (solid line) and 100 μM Pot1pN/d(GGTTAC) complex (dotted line) in 50 mM Na_2HPO_4, pH 7.5, 150 mM NaCl, 1 mM BME. Scans have been normalized against buffer-only scans to remove any spurious contributions from buffer artifacts.

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