Electronic Supplementary Material (ESI) for Soft Matter

SUPPLEMENTARY INFORMATION

Single Molecule Force Spectroscopy Reveals the Temperature-dependent Robustness and Malleability of a Hyperthermophilic Protein

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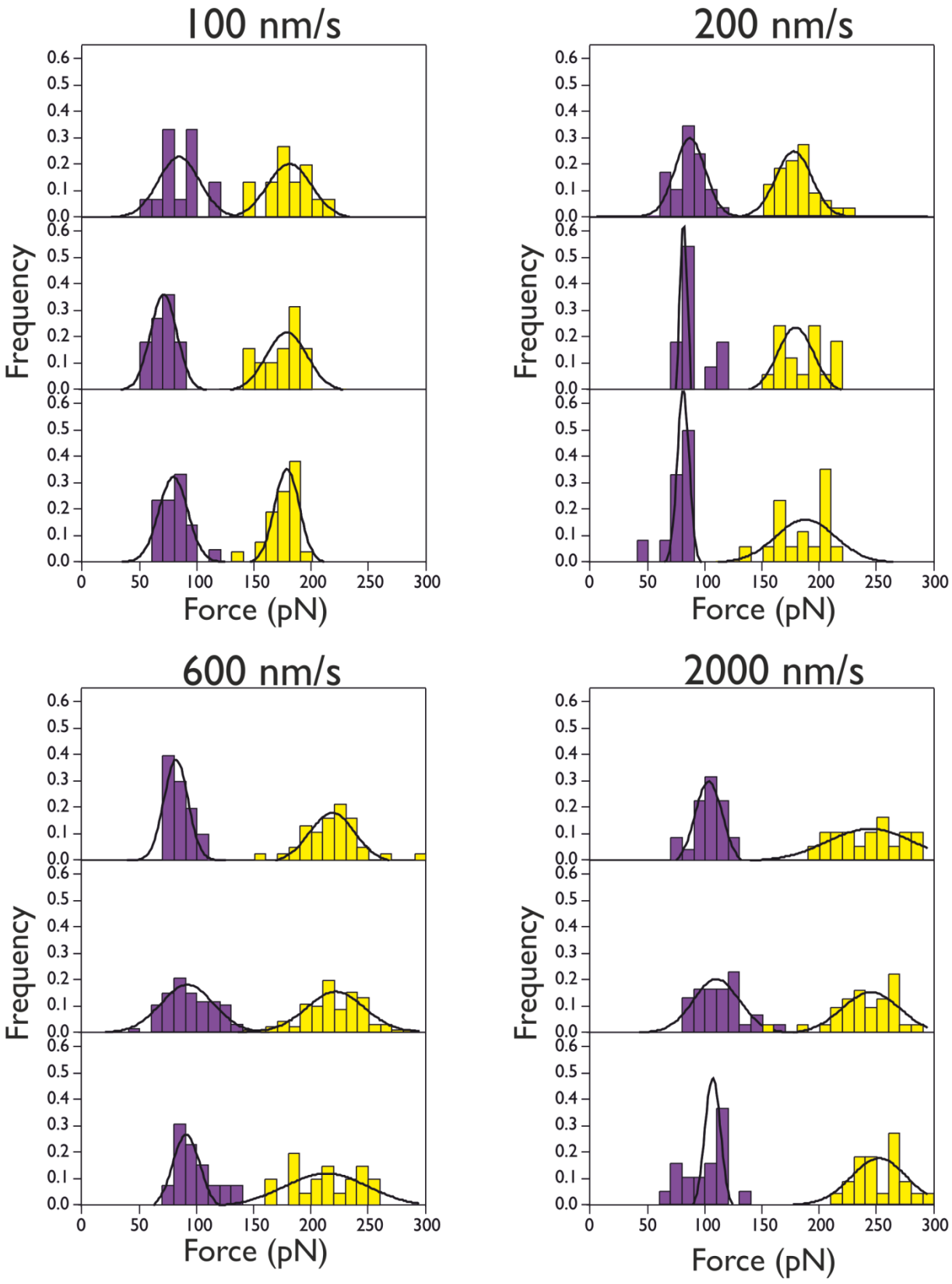

Figure S1: The unfolding force histograms for each experiment conducted at 5°C, showing the histograms for CSP events in purple and those for I27 in yellow, grouped by pulling velocity. Gaussian fits to the data provide a measure of the force distribution widths.

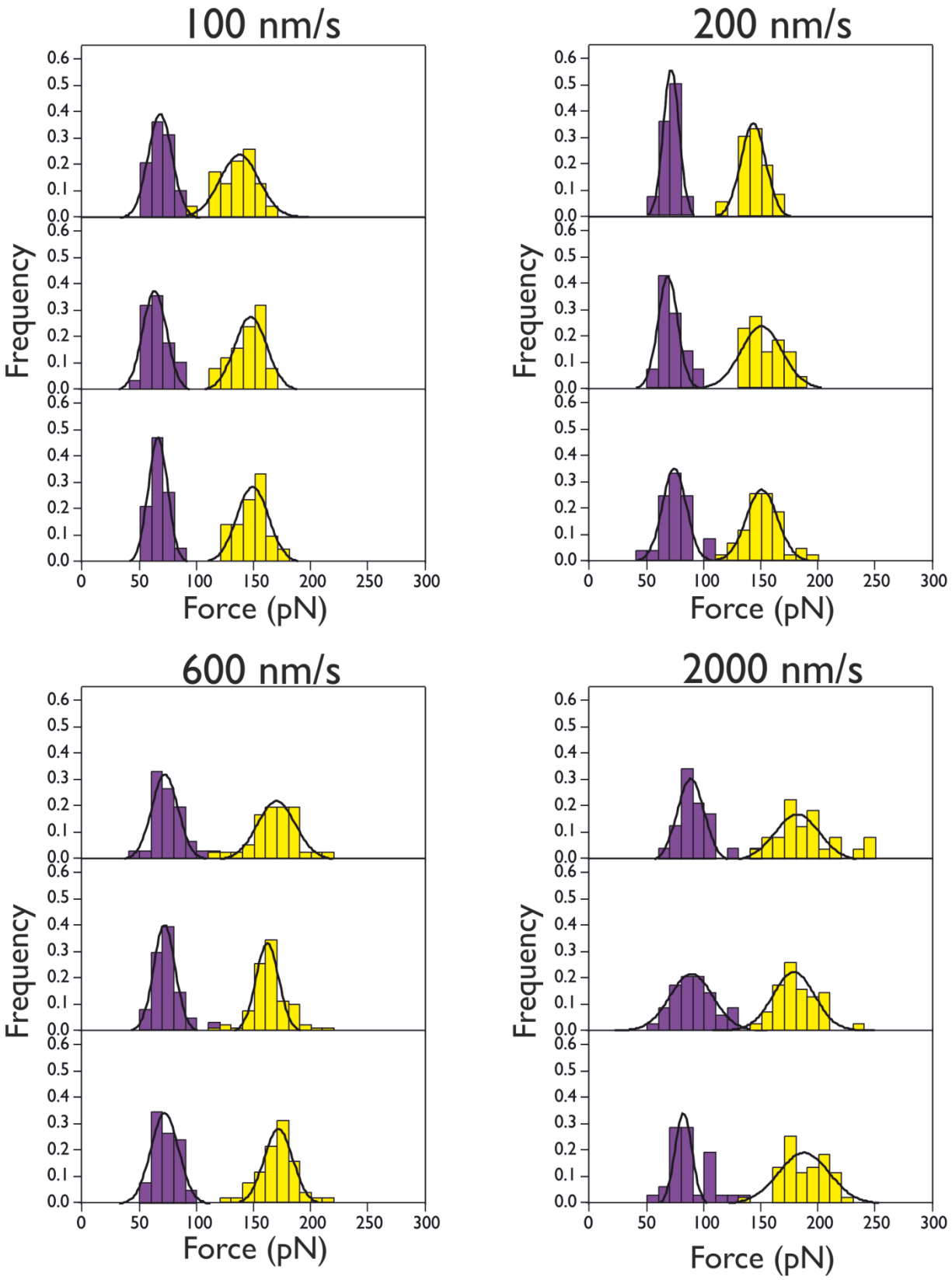

Figure S2: The unfolding force histograms for each experiment conducted at 23°C, showing the histograms for CSP events in purple and those for I27 in yellow, grouped by pulling velocity. Gaussian fits to the data provide a measure of the force distribution widths.

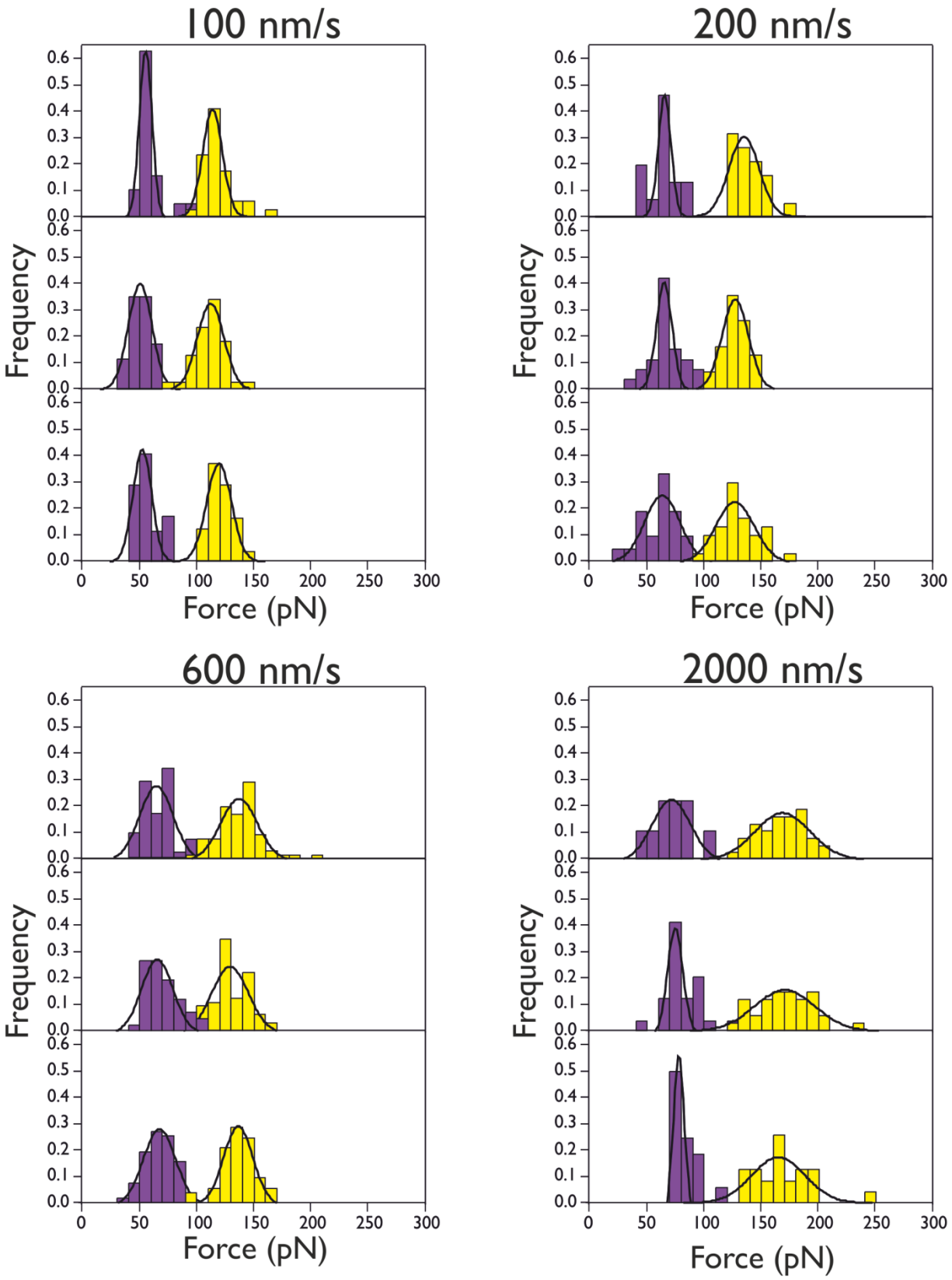

Figure S3: The unfolding force histograms for each experiment conducted at 40°C, showing the histograms for CSP events in purple and those for I27 in yellow, grouped by pulling velocity. Gaussian fits to the data provide a measure of the force distribution widths.

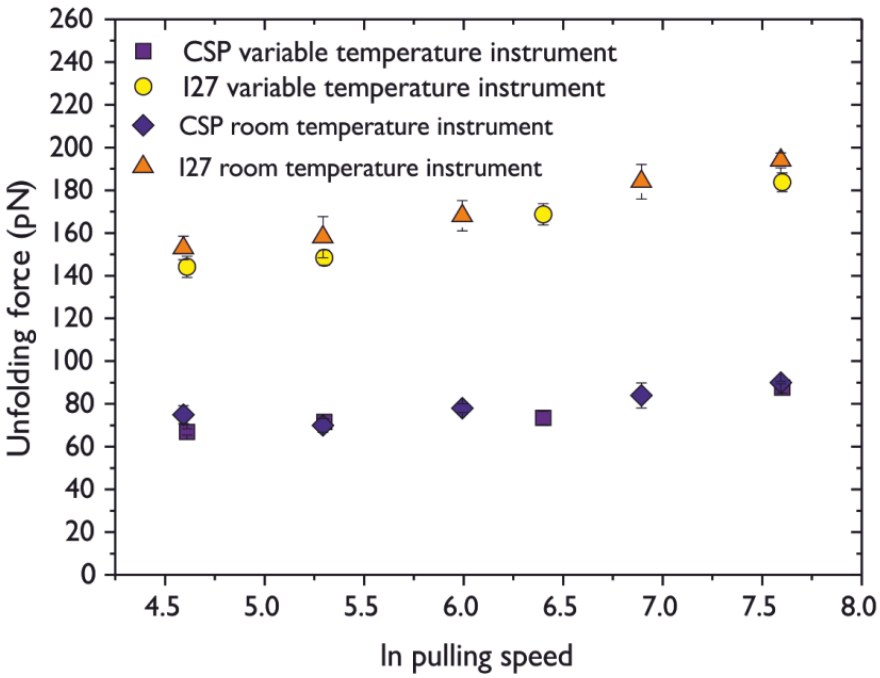

Figure S4: Comparison of the pulling speed dependence of the unfolding forces of I27 and CSP on the variable temperature instrument at 23°C used in this work and the room temperature instrument used to characterise the same sample under the same conditions, as previously reported in Journal of Physical Chemistry, B., 2013, **117**, 1819-1826.

Speed [nms^{-1}]	# Csp	# I27	Median unfolding force CSP [pN] (±SD)	Average [pN] (±SD)	Median unfolding force I27 [pN] (±SD)	Average [pN] (±SD)
100	15	15	85 (± 16)		172 (± 19)	
	11	19	74 (± 10)	80 (± 5)	179 (± 17)	176 (± 3)
	21	26	80 (± 12)		177 (± 13)	
200	27	31	88 (± 13)		182 (± 17)	
	12	16	87 (± 14)	86 (± 4)	186 (± 20)	183 (± 3)
	12	17	80 (± 11)		184 (± 22)	
600	10	37	85 (± 10)		218 (± 29)	
	13	20	95 (± 17)	91 (± 6)	212 (± 29)	216 (± 4)
	67	90	93 (± 20)		218 (± 28)	
2000	30	32	114 (± 19)		243 (± 29)	
	19	20	102 (± 19)	107 (± 6)	253 (± 23)	248 (± 5)
	20	18	107 (± 26)		247 (± 28)	

Table S1. Summary of mechanical unfolding data for (I27-Csp)$_3$-I27 at 5°C

Speed [nms^{-1}]	# Csp	# I27	Median unfolding force CSP [pN] (±SD)	Average [pN] (±SD)	Median unfolding force I27 [pN] (±SD)	Average [pN] (±SD)
100	19	52	68 (± 8)		139 (± 17)	
	28	25	66 (± 11)	67 (± 2)	147 (± 13)	144 (± 5)
	19	21	66 (± 7)		147 (± 13)	
200	14	36	72 (± 7)		144 (± 11)	
	14	22	70 (± 9)	72 (± 2)	151 (± 14)	149 (± 4)
	24	43	73 (± 12)		151 (± 16)	
600	31	35	77 (± 15)		170 (± 21)	
	61	78	72 (± 12)	73 (± 3)	163 (± 15)	169 (± 5)
	37	51	72 (± 10)		173 (± 16)	
2000	23	48	88 (± 16)		184 (± 26)	
	34	69	90 (± 17)	88 (± 3)	179 (± 18)	184 (± 4)
	31	43	85 (± 17)		188 (± 17)	

Table S2. Summary of mechanical unfolding data for (I27-Csp)$_3$-I27 at 23°C

Speed [nms^{-1}]	# Csp	# I27	Median unfolding force CSP [pN] (±SD)	Average [pN] (±SD)	Median unfolding force I27 [pN] (±SD)	Average [pN] (±SD)
100	19	34	53 (± 13)		114 (± 14)	
	17	38	54 (± 9)	54 (± 1)	111 (± 13)	115 (± 5)
	17	24	54 (± 13)		120 (± 10)	
200	15	19	64 (± 12)		137 (± 14)	
	26	31	66 (± 11)	65 (± 2)	127 (± 11)	131 (± 5)
	20	30	64 (± 16)		130 (± 17)	
600	41	65	67 (± 14)		138 (± 19)	
	41	63	66 (± 14)	67 (± 1)	128 (± 14)	134 (± 5)
	20	52	68 (± 12)		137 (± 14)	
2000	18	37	72 (± 16)		168 (± 20)	
	24	33	79 (± 15)	77 (± 5)	170 (± 24)	168 (± 2)
	16	23	80 (± 12)		167 (± 25)	

Table S3. Summary of mechanical unfolding data for (I27-Csp)$_3$-I27 at 40°C